DRUG UNIT

TEST METHODS

INDIANA STATE POLICE
DRUG UNIT
TEST METHODS

FOREWORD

The Drug Unit of the Indiana State Police Laboratory is routinely called upon to analyze drug evidence submitted by criminal justice agencies in the State of Indiana. These Test Methods are designed for the guidance of forensic scientists who support investigations of cases involving suspected drugs, both controlled and non-controlled substances. Its scope is limited to those compounds which are most frequently encountered such as narcotics, stimulants, hallucinogens, hypnotics, tranquilizers, diluents and materials from clandestine laboratories. These Test Methods are to be used in conjunction with Laboratory Division Policies.

The Drug Unit is staffed with forensic scientists in four laboratories and two technical supervisors for the North and South Zones. The North Zone serves the northern part of the State of Indiana with laboratories in Lowell and Fort Wayne, Indiana. The South Zone serves southern Indiana with laboratories in Evansville and Indianapolis. Each forensic scientist is required to have a Bachelors Degree in Forensic Science or a Natural Science with specific course requirements in Physics, General Chemistry, Organic Chemistry, Analytical Chemistry, and Instrumental Chemistry with laboratory classes. See Job Description.

Forensic Scientists in the Drug Unit participate in an extensive formalized training program under the supervision of a Drug Unit Supervisor. The training program begins with a general laboratory and safety orientation. The Drug Unit Training Program consists of several modules covering evidence handling, drug analysis and court testimony. Each drug module has a required reading list, practical exercises and examinations. Competency test samples are used to evaluate the progress of the trainee. Mock trials are given at the end of the Marijuana module and at the end of the General Drug modules, at a minimum. Upon a successful final mock trial, and the approval of the Division Commander, the trainee will be released to perform supervised casework.

These Test Methods documents provide a general approach to the examination of drug evidence. The Drug Unit procedures are a result of the contributions of a multitude of individuals and have been in place for several years. Instrumental operations are guided by the manufacturer's recommendations and standard practices in the discipline of drug analysis. All identifications made in the Drug Unit are made by direct comparison with known reference materials on the instruments in each respective laboratory under similar analytical conditions. The Drug Unit Test Methods are intended as a reference and are not necessarily all inclusive. Modifications to existing methods shall be validated and approved by the Division Commander. The degree of validation need not be exhaustive, but adequately demonstrate the purpose for which it was intended and must preclude or acknowledge false positives, false negatives and interferences. Alterations and/or deviations to procedures may be employed with approval from the respective Unit Supervisor.

INDIANA STATE POLICE
DRUG UNIT
TEST METHODS

TABLE OF CONTENTS

APPENDICES:

INDIANA STATE POLICE
DRUG UNIT
TEST METHODS

1. Evidence Handling

1.1. Scope: All evidence submitted for drug analysis shall be handled, stored, and sampled to preserve and protect the integrity of the evidence and to minimize the potential for cross contamination, destruction of evidence and personal exposure to drugs.

1.2. Precautions/Limitations: Forensic scientists shall take appropriate precautions to minimize contaminating, altering, or destroying the potential for additional future examination. Specific procedures shall be used when multiple exam requests are involved on an item.

1.2.1. Drug Only Examination Requests:
If drug analysis is the only requested or anticipated examination, then general routine precautions should be taken to minimize the potential for cross-contamination and personal exposure to drugs in the evidence. Lab coats and gloves shall be worn when handling any drug evidence. When handling large quantities of powder, masks shall be worn during sampling.

1.2.2. Biohazard Items:
For drug evidence suspected or marked as “Biohazard” (i.e. cigarette butts, body cavity seizures, evidence from toilet bowls, blood contaminated containers, etc.) the following procedures shall be used:

Forensic scientists shall wear gloves during the sampling process until the item is repackaged. After repackaging of the item, gloves shall be removed and hands washed prior to continuing with sealing the container.

Pens and papers should not be handled with potentially contaminated gloved hands during the sampling process. Pens that must be handled with gloves shall be washed with alcohol or appropriate cleanser prior to handling with bare hands.

1.2.3. Examinations Involving Other Disciplines: Items that need to be examined by another discipline, or multiple disciplines, may require consultation and/or collaboration to best process these items of evidence. With concern for dangerous drugs, collaboration and order of analysis should be considered. Routine tasks such as marking the evidence or sample collection can destroy or unnecessarily complicate evidence for those other disciplines. Consult with other units to discuss placement of markings on items and preservation of the evidence. There may be instances where adjustments need to be made to analytical procedures and/or general evidence handling.

1.2.4. Latent Prints:

If an item is submitted for drug and fingerprint analysis, the drug evidence and the container(s) shall be separated prior to submission to the laboratory as individual items. Special precautions may be necessary to preserve fingerprints during the drug sampling process.

In the event that an item cannot be separated into different items, a latent print examiner should be consulted prior to the examination to determine the best approach for the analysis and if marking of the evidence and/or its containers is appropriate. Adjustments to evidence handling procedures may be necessary to avoid the potential alteration or destruction of latent prints.

Items that have been previously examined by the latent print unit may have chemical exposure hazards. Discuss what chemicals were used with the latent print examiner and See 1.6.7 for the pertinent items.

1.2.5. DNA Examinations:

If an item is submitted for DNA analysis on the surface of the container and drug analysis on the contents, then the drug evidence and the container shall be separated prior to submission to the laboratory and submitted as individual items. Forensic scientists in the Drug Unit will not need special precautions for handling these drug sub-items to prevent contamination of the sub-item with the forensic scientist's DNA.

In the event that an item is submitted for Drug and DNA analysis, and cannot be separated, the investigator shall be consulted to determine which examination is of the highest importance and which analysis should occur first. Precautions are required during drug sampling to minimize the potential for contamination of an item with DNA from the forensic scientist.

1.2.5.1. When DNA sampling occurs first, the forensic scientists in the Drug Unit shall still comply with precautions during drug sampling for potential DNA analysis in the future.

1.2.5.2. Pens and sampling area surfaces shall be cleansed with appropriate cleaning material (e.g. methanol (MeOH), ethanol (EtOH), or a 5 - 10% bleach solution) prior to starting drug sampling, between sampling of another item with DNA analysis requested, and at the conclusion of sampling of these items.

1.2.5.3. During the drug sampling process the forensic scientist shall wear a disposable face mask and disposable gloves until the item is resealed.

1.2.5.4. Gloves shall be changed after each item is sealed and before sampling another item with DNA analysis requested.

1.2.5.5. If the external surface of gloves contacts skin or un-cleansed surfaces, then the gloves shall be replaced prior to handling items of evidence.

1.2.5.6. Other personnel present in the vicinity of the sampling area shall refrain from approaching or talking to the forensic scientist while this evidence is open.

1.2.5.7. At the conclusion of sampling of items with DNA analysis requests, the face mask and gloves shall be removed, disposed of in the appropriate disposal receptacle, and hands washed.

1.2.6. Document Examinations:
If both drug and document analyses are requested on an item of evidence, then the handling, marking and sampling must be accomplished to minimize damage to paper documents. This includes creation of additional indented writing impressions or damage to existing indented writing impressions.

Care shall be exercised not to damage handwriting on a document. Do not write on the envelope or container without consulting with a Questioned Documents examiner. Additionally, do not write on a paper that is lying on top of the evidence as this can add indented writing to the evidence.

1.3. Related Information:

1.3.1. Appendix 1 – Forms and Worksheets
1.3.2. Appendix 2 – Abbreviations
1.3.3. Appendix 3 – Definitions
1.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
1.3.5. Other Test Methods
1.3.5.1. Sampling
1.3.5.2. Weight Determination

1.4. Instruments: Heat sealers, fume hoods, fume absorbers or other ventilated work area.

1.5. Reagents/Materials: The varied nature of drug samples dictates that several types of containers can be utilized. Paper sacks, paper envelopes, plastic bags and glass bottles are suitable for most drug items depending on the physical make-up of the sample. Permanent markers shall be used for marking evidence. The use of gloves, face masks, tape and cleaning materials may be necessary.

1.6. Hazards/Safety:

1.6.1. Sharps, Broken Glass
1.6.2. Exposure to various drugs and chemicals
1.6.3. Biohazards
1.6.4. Bloodborne Pathogens Exposure Control Plan
1.6.5. Laboratory Safety Manual
1.6.6. Chemical Hygiene Plan
1.6.7. Safety Data Sheets (SDS)

1.7. Reference Materials/Controls/Calibration Checks: N/A

1.8. Procedures/Instructions: The following steps shall be accomplished during and/or after the transfer of evidence from the Evidence Clerk to the Forensic Scientist:

1.8.1. During Evidence Transfer: The identity of each item of evidence shall be verified by comparing the lab case number, item number, and evidence description, if possible, between the Request for Laboratory Examination Form and the actual evidence.

1.8.1.1. Verify that all evidence containers are properly sealed as per Laboratory Division guidelines and policies.

Note if any improper seals, suspected cross contamination between items, or tampering has occurred. If so, the Laboratory Manager shall be immediately notified and the situation documented in the analytical case notes.

1.8.1.2. The date and time of the transfer may be documented on the evidence, at the forensic scientists' discretion.

1.8.1.3. Numeric characters should be used as item numbers. Alpha characters should be used only as a means for identifying sub-items.(See Laboratory Evidence Policies)

1.8.2. After Evidence Transfer and During Sampling:

1.8.2.1. Verify the agency case number and item number marked on the evidence with Request for Examination Form.

1.8.2.2. Verify that all the items of evidence are properly described on the Request for Laboratory Examination form. Compare each item of evidence to the descriptions on the Request for Laboratory Examination form. Significant differences or conflicting information shall be correctly recorded in the case notes, and the correct information shall be updated in the Laboratory Information Management System (LIMS) case

information. It may be necessary to contact the contributor to advise of and/or resolve discrepancies.

1.8.2.3. The outer containers of evidence shall be marked with the initials of the forensic scientist, the lab case number and item number with leading zeroes.

1.8.2.4. An Examination Worksheet (General, Plant Material, or Tablets) shall be initiated for notes, observations, and conclusions during the analysis. (See 1.9.)

1.8.2.4.1. Each item of evidence received by the analyst shall be documented on an Examination Worksheet, if the item is in the analyst's personal custody. If practical, multiple items (or sub-items) may be combined on one worksheet or separated onto individual sheets.

1.8.2.4.2. Additional sheets, notes, and documentation are permitted for purposes of summarizing or clarifying analysis procedures and references. This may include summarization of multiple weights, references, etc. (See 1.9.1.1)

1.8.2.5. After sampling each item of evidence, each container shall be resealed and initials placed across the seal. Store the evidence in a secure temporary storage area until release of the case back to the evidence clerk.

1.8.2.6. Every effort should be made to avoid handling evidence repeatedly. The material should be sampled and immediately sealed. If necessary, the evidence may be closed and maintained in a secure temporary storage area until the analysis is complete.

1.9. **Records:** All evidence descriptions shall be described in detail on an Examination Worksheet (General, Plant Material, or Tablets). Details shall be sufficient to enable the forensic scientist, or other qualified individual, to identify the evidence at a later date.

1.9.1. The Examination Worksheet shall be labeled with the lab case number, item number with leading zeroes and date the worksheet was initiated.

1.9.1.1. Additional sheets for documentation are permitted and may be necessary to keep records and notes in a clear, readable and understandable form. These sheets shall be labeled with the lab case number, item number(s), and initialed.

1.9.2. Record a physical description for each item of the evidence in the analysis notes. This shall be documented on the Examination Worksheet.

1.9.3. Items that are administratively withdrawn shall be documented either on an Examination Worksheet or on the Request for Laboratory Examination form and include the relevant PEB information. (See also 1.11)

1.9.4. If the outer packaging of an item has been changed, this information shall appear on the Examination Worksheet. This does not include repackaging that is done because of the presence of dangerous drugs.

1.9.5. In the absence of a Request for Laboratory Examination form, submission form or secure documentation, the physical description of the evidence shall be recorded on the Examination Worksheet.

1.9.6. The Examination Worksheet shall be dated and initialed / signed.

1.9.7. Common abbreviations or those that are found on the approved abbreviation list are acceptable for use in analytical notes. Unapproved abbreviations shall not be used.

1.9.8. The date of each test or observation shall be recorded in the case notes.

1.9.9. Photographs, if taken for the purposes of analysis, shall be included in the notes or printed and attached as part of the analysis. Proper identifiers and scales shall be in the content of the photograph for identification.

1.10. Interpretations of Results: Proper evidence handling is determined by an intact and sufficient seal that prevents loss, cross-contamination or deleterious change of the evidence in a container. Markings shall be on the container and the seal for the purposes of identification and security.

1.11. Report Writing: All evidence shall be described as being “sealed” in the report, unless it is the forensic scientists’ opinion that there is a question regarding the integrity of a seal. In the event that a seal may be insufficient to prevent loss, cross-contamination or deleterious change, the word “sealed” shall be removed from the evidence description on the Certificate of Analysis and the Request for Laboratory Examination form.

Items that clearly do not meet Drug Unit submission guidelines and do not have sufficient justification, or approval, for analysis may be administratively withdrawn and one of the following statements shall be used:

Item 001 - the request for examination was administratively withdrawn as per Indiana State Police Physical Evidence Bulletin XX, section X.

or

Item 001 was not examined as per Indiana State Police Physical Evidence Bulletin XX, section X.

Any item administratively withdrawn or not examined may be resubmitted and analyzed at a later date if/when sufficient justification is given to warrant analysis. Marked pharmaceutical tablets and capsules not examined as per the PEB should be reported as reference identifications. Exceptions to the Physical Evidence Bulletins (PEB) shall be initialed by the authorizing person on the submission sheet, or in the absence of the submission sheet, in LIMS under the request information. A Unit Supervisor or Laboratory Manager should be advised of items that are administratively withdrawn.

Cases that have been looked up in MyCase or DoxPop can be administratively withdrawn without contacting the contributor or prosecuting attorney, if the suspect(s) has been sentenced, the charges dismissed, or the suspect(s) has entered pre-trial diversion. The source of the information, cause number, date the information was looked up, and the disposition of the case shall be stated on the Certificate of Analysis (see 2019 Marijuana Reporting Directive 19/Lab Div/016 on SharePoint).

1.12. References:

1.12.1. Lab Policies
- **1.12.1.1.** QA Manual - Lab Case Notes
- **1.12.1.2.** QA Manual - Evidence Handling
- **1.12.1.3.** Laboratory Evidence Policies

1.12.2. Laboratory Physical Evidence Bulletins (PEB's)
- **1.12.2.1.** Clandestine Laboratory Samples Submission
- **1.12.2.2.** Evidence Handling
- **1.12.2.3.** Drug Submissions

2. Sampling:

2.1. Scope: This section is intended to provide procedures for the sampling of items of evidence suspected to contain controlled substances and/or other drugs.

2.2. Precautions/Limitations: The basis of sampling is that the composition found in the sample removed for analysis represents the composition of the material from which it was taken. The forensic scientist shall ensure that the sampled material represents the item(s) by making careful visual examinations and considering the homogeneity among drug packaging (bags, packets, etc.) and its contents.

2.2.1. General: When a single unit is to be analyzed, one sample is sufficient if the material appears to be homogeneous. If the material is not homogeneous, additional samples may be necessary to represent the item as a whole or steps may be taken to make the sample homogeneous.

When multiple inner packages containing similar materials as a single item are submitted for examination, a sufficient number of individual containers (bags, packets, etc.) shall be examined so that the total net weight of the contents in that case will meet and/or exceed the requirements of a particular criminal charge (e.g. weight limits associated with possession or dealing charges, etc.).

Enough samples shall be analyzed to meet the highest weight threshold for that case. It is not always necessary for weight thresholds to be met for each item if enough samples have been taken in other items (except for cases requesting aggregate charges of heroin).

If the total net weight of the contents or gross weight of the containers and the contents, within an item is less than the legal weight requirements to elevate the criminal charge, a minimum of one sample shall be taken for analysis. It is up to the analyst to determine if there is sufficient sample to test a specific item.

The potential presence of a controlled substance, legislative requirements, weight thresholds, and measurement uncertainty must be considered when sampling.

2.2.2. Items containing large numbers of containers: For items containing large numbers of similar containers (foil packets, knotted plastic bags, etc.), a hypergeometric sampling strategy may be used (See 2.12.1 and 2.12.2). This allows a portion of the containers to be analyzed and a statistical inference to be made about the entire item as a whole. When a hypergeometric sampling strategy is chosen, a sufficient number of samples will be examined to meet, or exceed, a 95% confidence that 90% are positive and satisfy the requirements of the criminal charges. The date and time of this sampling shall be recorded in the case file.

2.2.3. Bulk materials: Bulk materials (e.g., bricks of compressed powder, bales of plant material) should be broken or cored to obtain a representative sample. Depending on the size of the material, samples from several locations may be required to obtain a representative sample. The locations from which the samples were obtained shall be described in the analysis notes. A drawing or description is sufficient.

2.2.4. Tablets and Capsules: The sampling scheme used for general unmarked drugs is not required for samples that appear to be pharmaceutical preparations, which have unique physical identifiers present and are clearly visually consistent with each other. Generally, a single sample may be taken for a given type of drug. Individual whole tablets and capsules are to be treated as separate samples and cannot be combined for analysis. It may be necessary to sample an entire tablet or capsule for low dosage preparations.

2.2.5. Residues: Residues are samples which are either too small to be weighed accurately or that which remains after the bulk has been removed. Residues can be sampled by mechanical means (e.g. shaking or scraping) or chemical means (e.g. rinsing with solvent). Case notes shall reflect the method by which the sample was removed. The method of sampling can be documented either on the preparation line or in the item description of the Examination Worksheet. The evidence description can state that the item is a "residue".

2.2.6. When possible, a sample should be removed while leaving a portion of the residue intact.

2.2.6.1. For items containing multiple sub-items with residues, a minimum of one sample will be taken for examination. It is up to the analyst to determine if there is sufficient sample or reason to test the remaining items.

2.2.7. Weighing: See Weight Determination Test Method (See 3.2)
The uncertainty associated with the weight of individual items may affect the number of samples that need to be examined.

2.3. Related Information:

2.3.1. Appendix 1 – Forms and Worksheets
2.3.2. Appendix 2 – Abbreviations
2.3.3. Appendix 3 – Definitions
2.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
2.3.5. Other Test Methods:
2.3.5.1. Weight Determination
2.3.5.2. General Drug Analysis

2.4. Instruments: N/A

2.5. Reagents/Materials: General laboratory supplies: spatulas, scissors, scalpels, tape, pens, methanol (MeOH), chloroform ($CHCl_3$).

2.6. Hazards/Safety:

2.6.1. See Evidence Handling
2.6.2. See Safety Policies
2.6.3. Potential chemical exposure to methanol, chloroform and clandestine laboratory chemicals.

2.7. Reference Materials/Controls/Calibration Checks: N/A

2.8. Procedures/Instructions:

2.8.1. A small representative sample shall be removed from each item of evidence (See 2.2 for sampling considerations). A minimal amount of sample should be removed for anticipated analysis. No more than one-half of the original material should be routinely sampled. If an entire sample is removed for analysis, the autosampler vial containing the sample shall be returned to the evidence. The date the vial was returned to the evidence shall be documented on the Examination worksheet.

2.8.2. Cigarette butts, cigarettes, and loose plant material all in one container that are potentially cross-contaminated may be treated as one item and one sample may be taken for analysis. The analyst shall note what was weighed, sampled and analyzed. The Prosecutor needs to approve the reporting of gross weights that are over a statutory weight limit.

2.8.3. Reference Identification: Pharmaceutical identifiers on tablets and capsules (markings, color, shape, and other characteristics) shall be compared to published references. Examples of published references are The Physicians' Desk Reference, The Logo Index (printed or computer version), Ident-A-Drug, Med Scan, Drug Identification Bible, Pill Box, Drugs.com, generic drug company lists, Poison Control, and pharmaceutical company internet sites.

2.8.3.1. If reference identification is used, there are instances where the removal and weighing of a sample may not be necessary.

2.8.4. Tablets and Capsules: It is advisable to sample half or less of a tablet or capsule contents, leaving the remaining portion in the evidence for future examinations, if possible. It may be necessary to sample an entire tablet or capsule in low dosage preparations. Supervisory approval shall be required to consume whole tablets or capsules. When an entire tablet and/or capsule is sampled, the autosampler vial containing the sample shall be returned to the evidence.

2.8.4.1. Marked Pharmaceutical Tablets with weight thresholds: At a minimum, one tablet shall be fully examined. It is permissible to perform reference identification on the remaining tablets. (See 2.11.1 for reporting)

Partial Tablets with Whole Tablets: If one of the whole tablets is analyzed, the reference identification can include the partial tablets if the partial tablets are visually consistent or resemble the whole tablet(s). The partial tablets can also be grouped together and referred to as "Not examined."

Partial Tablets: Reference identification cannot be used as a second test for identification of partial tablets unless full markings are present. If the item only contains partial tablets that are visually consistent with one another, only one partial tablet needs to be examined regardless of whether or not a weight threshold applies. The remaining partial tablets can be reported as not examined or not analyzed. It is the examiner's discretion whether or not to count the number of partial tablets.

2.8.4.2. Marked Pharmaceutical Capsules with weight thresholds: At a minimum, one capsule shall be fully examined. It is permissible to perform a reference identification on the remaining capsules, if the weights (either taken individually or the calculated average) of the examined capsules are consistent with each other.

2.8.4.3. Illicit Capsules with weight thresholds: Enough capsules shall be analyzed separately and fully to meet or exceed the weight threshold.

2.8.4.4. Marked Illicit Tablets with weight thresholds: Enough individual tablets shall be analyzed to meet weight thresholds.

2.8.5. Marked Pharmaceutical Sublingual Films: If multiple sublingual films are present in an item, one film shall be fully analyzed, at a minimum. A reference identification of the remaining films can be performed if full markings are present. The weight of the examined and unexamined films shall be documented on the Examination Worksheet, but may be omitted from the Certificate of Analysis.

2.8.6. Items containing specimens of multiple colors (plastic bags, capsules, etc.): One specimen of each color does not need to be examined. However, enough specimens shall be analyzed to meet and/or exceed weight requirements.

2.8.7. The sample should be transferred and stored in a disposable test tube and/or disposable analysis vial marked with the lab case number and item number.

2.8.8. Once the sample has been collected, the sample tube and/or vial shall be fitted with a closure, such as a stopper, cork, cap or parafilm, etc. to protect the sample from loss or contamination, except for during analysis.

2.8.9. Unused disposable sample tubes and/or vials shall be stored and handled in a manner to protect them from contamination.

2.9. **Records:** The examination documentation shall be of sufficient detail to describe the contents of the item undergoing examination including all levels of interior packaging (number of inner packages, etc.), the creation of any sub-items and all weights measured.

2.9.1. Specifically define what was weighed, sampled and tested and what, if anything, was only weighed. Tablets and capsules are to be treated as separate samples. Detail what tests were conducted on which items and/or sub-items.

2.9.2. Sample preparation shall be described in the analytical notes. This may include the method of sampling, description or depiction of where the sample was taken and/or other steps taken to prepare the sample for analysis.

2.9.3. If an additional sample of an item is taken and testing is performed, the notes and data shall specify which test results are for which sample. The notes shall also indicate if the additional sample was combined with the original sample.

2.9.4. When the hypergeometric plan is utilized, the analyst shall document the confidence level on the Examination Worksheet and in the report.

2.9.5. Reference identifications shall be recorded on the analysis sheet and shall include information such as the name and version of the reference, active ingredients, size of dosage unit (mg of drug), and control status (including schedule number), at a minimum. The trade name or drug generic name, prescription (Rx) or over the counter (OTC) status, and drug company name should also be documented.

If the tablet or capsule contains multiple salt forms of the same active ingredient, each individual component does not need to be documented. The active ingredient(s) in the tablet or capsule can be referred to as mixed salts. For example, “mixed Amphetamine salts”.

2.10. Interpretations of Results:

2.10.1. Non-Statistical (Administrative) Sampling: A non-statistical, or administrative, approach is intended to satisfy the requirements of a specific charge. Unless all items (or containers) are weighed and individually analyzed, no inference can be made regarding the contents of any unexamined items.

Tablets and Capsules: No inference can be made regarding the contents of any unexamined tablet(s) or capsule(s). However, reference identification may be used to indicate the contents of the remaining unexamined tablet(s) or capsule(s).

2.10.2. Statistical Sampling: A statistical approach allows a specific portion of containers within an item to be examined and permits a statistical inference regarding the remaining unexamined containers (or items). This method will be used to meet, or exceed, a 95% confidence level that at least 90% of the containers within the item are the same. (See 2.2.1 and 2.2.2)

If statistical sampling reveals the presence of a heterogeneous population, non-statistical sampling shall be used. Enough samples to meet and/or exceed the weight threshold(s) shall be taken.

2.11. Report Writing: If an item of evidence contains several containers (example- Item 001 contains four plastic bags of vegetation), then these can be sub-itemized. If the sub-itemizing is listed in the description on the Certificate of Analysis, then the same sub-itemizing shall be in the results.

2.11.1. Non-Statistical Sampling Results: For those items where samples have been taken, examined and conclusions reached, the reports shall contain information regarding what was examined and the weight of the examined material. Additional statements containing information regarding items that were not examined and the net or gross weight of the unexamined items shall also be reported, if the weight of the unexamined items could affect the charges. (See also General Drug Analysis 4.11)

The report must specify what was examined and not examined if the remaining unexamined items could be resubmitted for additional analysis.

Generally this will apply to the non-statistical sampling of multiple inner packages where sufficient samples are taken to meet statutory weight requirements of specific criminal charges. Statements that do not apply to the item results may be omitted.

For consistency in reporting, the below listed examples, or similar verbiage, shall be used, unless they need to be adjusted for accuracy.

Descriptions and results in reports shall be clear and maintain a consistent format.

For example:

Items 001(A-C) were found to contain X, a controlled substance.
The net weight of items 001(A-C) was X grams.
Items 001(D-F) were not examined and had a (net/gross) weight of X grams.

or

Item 001: Thirty-seven (37) packets were examined and each found to contain X, a controlled substance, and had a total net weight of X grams.

The remaining sixty-three (63) packets were (visually/not) examined and had a (net/gross) weight of X grams.

or

If all of the samples in an item have been examined, the item may be reported as per 4.11. For example:

Item 001 was found to contain X, a controlled substance.
The net weight of item 001 was X grams

Tablet and Capsule Reporting Example:

Item 001: One tablet (capsule) was examined and was found to contain X, a controlled (or non-controlled) substance and had a net weight of X grams.

The remaining tablets (capsules) were visually examined and had a net weight of X grams. Reference(s) indicated the presence of X, a controlled (or non-controlled) substance. No confirmatory analysis was performed on the remaining tablets (capsules).

In instances where a reference identification is performed but is inconsistent with the analytical results, the remaining tablets may be reported as “not analyzed”.

2.11.2. Statistical Sampling Results: The conclusion reached shall be clearly stated with respect to what inference could be drawn from the analysis of a multiple unit population in the case notes. If a statistical sampling plan (hypergeometric) is used, it is statistically correct to infer that the results of the items examined include the unexamined items. (e.g. 29 bags out of 100 were examined and found to contain Cocaine. The results can be reported as “found to contain Cocaine, a controlled substance”, and the total weight reported, as appropriate.) Refer to the tables found in 2.12.2.

When statistical sampling is used, the following statement shall be added to the results section of the Certificate of Analysis. “This result is based

on statistical sampling that meets or exceeds a 95% confidence level that 90% of the containers are positive."

2.12. References:

2.12.1. Methods of Analytical/Sampling Seized Drugs for Qualitative Analysis: Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations 2010-Jan-29, Part IIIA.

2.12.2. Guidelines on Representative Drug Sampling, European Network of Forensic Science Institutes (ENFSI) Drugs working Group

2.12.3. Indiana Criminal Code 35-48

2.12.4. Indiana Criminal Code 35-48-1-25

2.12.5. Indiana Criminal Code 16-18-2-199

2.12.6. United States Criminal Code Title 21 Section 801

2.12.7. United States Criminal Code Title 21 Section 802 (41) (A and B)

2.12.8. United States Criminal Code Title 21 Section 812

2.12.9. United States Criminal Code Title 21 Section 813

2.12.10. United State Criminal Code Title 21 Section 353(b) (1)

3. Weight Determination:

3.1. Scope: Forensic Scientists conduct examinations on evidence suspected to contain controlled substances and/or other drugs. In addition to qualitatively determining what drug or drugs are present, the forensic scientist routinely measures the weight of the drug or material present in the evidence submitted. (Note: Here the terms “mass” and “weight” are used interchangeably.) The weight of the evidence, whether it is plant material, powder, tablets, capsules, a rock-like substance, etc. is specifically being measured. It is recognized that in some instances the results of these measurements and their associated uncertainties have an effect on criminal charges.

3.2. Precautions/Limitations: In some situations, the mass or weight may not be recorded or reported. For example, items that do not register on a balance with a readability of 0.01 gram and/or in situations where the combination of the weight and uncertainty is equal to zero, or results in a negative number, may be described as a residue, or suspected residue, and therefore a weight is not recorded.

If the weight of the controlled substance in an item is significantly less than the packaging of the item, or the medium containing a drug, a weight does not need to be reported. If the sample is intended to be consumed, the weight is to be recorded as a net weight (unmarked capsules are an exception to this). If the sample is not intended to be consumed, it is to be recorded as a gross weight. Weights shall be recorded in the analyst’s notes regardless of reporting.

The uncertainty of weight measurements may affect sampling procedures and it may be necessary to adjust sampling and weighing methods to reduce the overall measurement uncertainty of an item or items. (See the Drug Unit Uncertainty Documents)

Some items cannot be accurately weighed due to their condition, such as removing all of a sticky tar-like substance from its packaging, or removing powder from tape. In those instances, the forensic scientist would not record a measurement and record the condition of the material in their case notes. (See 1.9.2)

It is important that balances are functioning properly prior to obtaining weight measurements.

3.3. Related Information:

3.3.1. Appendix 1 – Forms and Worksheets
3.3.2. Appendix 2 – Abbreviations
3.3.3. Appendix 3 – Definitions
3.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
3.3.5. Other Test Methods

3.3.5.1. Drug Unit Uncertainty of Measurement Statements
3.3.5.2. Sampling Test Method

3.4. **Instruments:** Measurements will be made using the laboratory balances of various manufacturers and models, and will generally be an electronic top-loading balance with a readability of 0.01 gram. In some instances, there will be a need for a higher capacity balance for large items of evidence or an analytical balance may be used for items requiring a greater sensitivity with different readabilities. The forensic scientist shall use each balance in accordance with the manufacturer recommendations found in the balance user manuals.

3.5. **Reagents/Materials:** Weigh boats, weigh paper or other container may be used during the weighing process.

3.6. **Hazards/Safety:** Forensic scientists shall comply with the Chemical Hygiene Plan, and the Laboratory Safety Manual. Precautions should be taken to minimize the potential for personal exposure to drugs, hazardous chemicals and potential biohazards. Gloves shall be worn during the weighing process of evidence handling.

3.7. **Reference Materials/Controls/Calibration Checks:**

3.7.1. The performance of all balances shall be verified, evaluated and their respective uncertainties calculated prior to use in case work.

3.7.2. Reference Standards (weights):

3.7.2.1. National Institute of Standards and Technology (NIST) traceable weights shall be used to verify the calibration status of the balances.

3.7.2.2. Weights used to check balance accuracy shall be re-certified by a qualified vendor every three years, at a minimum. (Effective December 31, 2011). Vendor and documentation specifications shall be maintained on a network drive.

3.7.2.2.1. Any weight found to be outside the manufacturer specified range of tolerance shall be repaired and returned to acceptable tolerances, if possible. If a weight cannot be adjusted or repaired, it shall be marked and retired from service.

3.7.2.3. Reference Standard weights of 1 gram, 5 grams and 30 grams shall be used, at a minimum, for verification of small capacity balances.

3.7.2.4. High capacity balances shall be verified with standard weights of 30 grams and 10 pounds, at a minimum.

3.7.2.5. Reference Standard Weights shall be stored in a box or closed container.

3.7.2.6. Reference Standard Weights shall be handled with tweezers, or with gloves or other protective material to keep the weights from accumulating contaminants.

3.7.3. Acceptable measurements for reference standard weight sets:

3.7.3.1. High Capacity balances capable of reading to 1 gram shall be within +/- 1 gram of the reference standard weight used.

3.7.3.2. Balances capable of reading to 0.1 gram shall be within +/- 0.1 gram of the reference standard weight used.

3.7.3.3. Balances capable of reading to 0.01 gram shall be within +/-0.01 gram of the reference standard weight used.

3.7.3.4. High Capacity balances capable of reading to 0.005 pound shall be within +/- 0.005 pound of the reference standard weight used.

3.7.3.5. High Capacity balances capable of reading to 0.0005 pound shall be within +/- 0.0005 pound of the reference standard weight used.

3.7.3.6. Analytical Balances capable of reading to 0.0001 gram shall be within +/- 0.0001 gram of the reference standard weight being used.

3.7.4. Calibration Checks:

3.7.4.1. The balance calibration shall be verified by the analyst before and after evidence sampling. (See 3.9.1 and 3.9.2)

3.7.4.2. A measurement outside the acceptable limits indicates a possible problem. Re-run the verification procedure after checking the balance and weight conditions (vibration, level of balance, drafts, cleanliness of weight, etc.). If the balance does not meet acceptable measurements, then the balance will be identified as “out of service” and the supervisor or laboratory manager shall be notified.

3.7.4.3. Balances shall be calibrated/serviced/verified annually by a qualified external vendor, demonstrating the balance is working

properly by using standard weights traceable to NIST (National Institute of Standards and Technology). The methods and specifications for the external calibration of the balances shall be determined by the vendor performing the calibration service. Vendor and documentation specifications shall be maintained on a network drive.

3.8. Procedures/Instructions:

3.8.1. Drug evidence should be weighed prior to analysis using an appropriate type of balance, except for the following examples. (See Sampling 2.2.1, 2.9.1 and 2.11.1)

3.8.1.1. Liquids: If required, the approximate volume can be recorded (e.g. Clandestine Laboratory samples). Weighing liquids is not an accurate measurement and this shall not be performed.

3.8.1.2. Residues: Examples include spoons, pipes, straws, bags, etc.

If evidence has not been opened (e.g. visually examined tablets or administratively withdrawn), the items do not need to be weighed unless the item is plant material where a weight threshold has already been met.

3.8.2. Ensure the balance is on, level and reads zero.

3.8.2.1. Tare the balance, if necessary.

3.8.3. Verify that the balance is working properly as per 3.7.

3.8.3.1. Document the satisfactory balance calibration verification on the Examination Worksheet. (see 3.9.1)

3.8.3.2. If the calibration verification check is unacceptable, see 3.7.4.2.

3.8.4. Place suitable container (see 3.5) on the pan when appropriate.

3.8.5. Re-tare the balance.

3.8.6. Place the sample in the tare container, or on the pan, as appropriate.

3.8.6.1. Record the value displayed on the balance when and where appropriate as per 3.7.3.

3.8.6.2. Calculate and record the associated uncertainty in the case notes. The uncertainty of each individual measurement shall be documented in the case notes. The case notes shall specify that the uncertainty of measurement is for each weight if multiple

weights are recorded. The total uncertainty for weights that are going to be combined shall also be documented in the case notes. (See uncertainty statement.)

3.9. Records:

3.9.1. Each balance used for casework in the laboratory will have a calibration verification log. Verifications shall be recorded once per month at a minimum for the purpose of calculating and maintaining the uncertainty of measurement.

3.9.2. The balance calibration verification shall be documented on the Examination Worksheet with a unique identifier, or the serial number, of the specific balance(s), the weight set(s) used and the date of the verification.

Balance checks shall be recorded on the Examination Worksheet before and after sampling. If verifications are performed on different dates, both dates shall be recorded on the Examination Worksheet.

3.9.3. All numerals displayed by the balance during calibration verification shall be recorded in the calibration verification log.

3.9.4. All numerals displayed by the balance during the sampling process shall be recorded on the Examination Worksheet. All weights shall be recorded as a net or gross weight and shall be recorded on the Examination Worksheet.

3.9.5. Marijuana items greater than ten (10) pounds shall be recorded in both grams and pounds in the analytical notes and reported on the Certificate of Analysis.

3.9.6. Weighing of Capsules:

Marked Pharmaceutical Capsules with reference identification: The net weight includes the capsule and the contents.

All Other Capsules: The net weight is of the contents only and the gross weight is the combination of the capsules and their contents.

3.9.7. All weights used to achieve and/or exceed weight limits to meet a particular criminal charge shall be recorded as a net weight. The remaining weight(s) may be recorded as a gross weight.

3.9.8. When adding weights of multiple packages, the total net or total gross weight shall be in the notes.

3.9.9. A record shall be kept of the calibration status of the reference standard weights and/or weight sets.

3.9.10. In instances where several samples are taken but weighed simultaneously, the Examination Worksheet shall indicate the method used to take this measurement (test tube, grid, etc.). See Appendix 3 for definition.

3.9.11. If a weight is to be reported in pounds, no more than two decimal places shall appear on the report.

3.10. Interpretations of Results: The Drug Unit has conducted studies to estimate the uncertainty associated with weight measurements. For the Expanded Uncertainty the Drug Unit recognizes k=2 as an uncertainty window with approximately a 95% confidence for a single measurement. (See Uncertainty Statement and updates.)

3.11. Report Writing:

3.11.1. All weights shall be reported as net or gross weight and to the proper decimal accuracy not to exceed the readability of the balance used.

3.11.2. Weights from balances with different readabilities shall not be combined for total weight reporting.

3.11.3. Weights of multiple items may be combined to report a total weight only if the weight types are the same. If the weights are added together, it shall be reported as a "total" weight. It is not appropriate to mix net and gross weights together for a total weight.

For example: You cannot add an item with a net weight of 0.20 gram and an item with a gross weight of 0.20 gram and report a total net or gross weight of 0.40 gram. If both weights are recorded as net weights, or both are gross weights, then they can be combined for a total weight.

3.11.4. If the mass of any item is less than the readability of the balance, the weight may not be reported. The item may be described as a residue, or suspected residue, on the Certificate of Analysis.

If the total uncertainty of multiple items is large or may cause the weight range to drop into negative numbers, procedures should be taken to reduce the uncertainty, if possible and practicable.

3.11.5. In cases where a relevant sample weight cannot be obtained due to its condition, a weight may not be reported.

3.11.6. It may not be necessary to report weights of non-controlled substances.

3.11.7. Marijuana items greater than ten (10) pounds shall be reported in both grams and pounds. If applicable, it is advisable to report the pound equivalents for individual items that are approximately one pound or more for clarity in reporting.

3.11.8. The measurement uncertainty shall be reported when the uncertainty causes the weight to drop below a statutory threshold and shall be reported as +/- the total uncertainty "at a coverage probability of 95.45%".

3.11.9. If the weight of an item is 0.00 gram but the item is not described as a residue, then the weight shall be reported as "less than 0.01 gram".

3.12. References:

3.12.1. Ballard and Roskowski, Uncertainty Statements – Drug Unit, 2010

3.12.2. Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Supplemental Document SD3 Measurement Uncertainty for Weight Determinations in Seized Drug Analysis, 2010-01-28

3.12.3. Drug Unit Uncertainty of Measurement Statements (SharePoint)

4. General Drug Identification:

4.1. Scope: This Test Method is intended for the guidance of laboratory personnel who support investigations of cases involving suspected drugs, both controlled and non-controlled substances. Its scope is limited to those compounds which are most frequently encountered such as narcotics, stimulants, hallucinogens, hypnotics, tranquilizers, diluents and materials from clandestine laboratories.

Techniques for analysis of drug samples are classified into three categories by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) based on their discriminating power for identification of drugs. Testing procedures selected should give useful (positive) information for suspected drugs for items being examined based upon an initial appraisal of the sample. The currently accepted analytical methods used in this laboratory are broken down into three categories:

Category A: Those that provide structural information:

Infrared Spectroscopy
Mass Spectrometry

Category B: Methods that provide a high degree of selectivity:

Gas Chromatography
Pharmaceutical Identifiers (Reference Identification)
Thin Layer Chromatography
Macroscopic Exam (Cannabis only)
Microscopic Exam (Cannabis only)

Category C: Those that provide presumptive information:

Color Tests
Ultraviolet Spectroscopy
Melting Point
Polarimetry

Scientifically sound practices require the use of multiple techniques. It is the responsibility of the forensic scientist to identify the sample and to provide requested information about the sample. These Test Methods specify the minimum testing procedures required for the identification of controlled and non-controlled substances. A minimum of two independent testing procedures is required for identification of substances. This generally includes one preliminary (Category A, B or C) and one confirmatory testing procedure (Category A). Further testing procedures may be performed at the discretion of the analyst. It is the responsibility of a forensic drug analyst to identify controlled substances and other drugs that may be present in evidence samples. If the result of a preliminary test indicates the presence of a controlled substance (even a weak indication), steps shall be taken to attempt to confirm the controlled substance.

When a mixture contains multiple controlled substances, at least one controlled substance shall be identified, if possible. Unless charges could be affected, other controlled substances may be indicated only, at the analyst's discretion. Attempts to identify the primary components should be made.

4.2. Precautions/Limitations: These Test Methods do not include every possible technique or procedure. Forensic Scientists must exercise sound analytical judgment in choosing the appropriate procedure for the circumstances. Insufficient material or concentration within submitted samples may preclude an examination and/or identification. New methods, or modification of existing methods, must be accepted scientific techniques and apply to the individual sample.

4.3. Related Information:

- **4.3.1.** Appendix 1 – Forms and Worksheets
- **4.3.2.** Appendix 2 – Abbreviations
- **4.3.3.** Appendix 3 – Definitions
- **4.3.4.** Appendix 4 – Drug Unit Reagent Preparation Manual
- **4.3.5.** Other Test Methods
  - **4.3.5.1.** Marijuana Test Method
  - **4.3.5.2.** Color (Spot) Tests
  - **4.3.5.3.** Thin Layer Chromatography
  - **4.3.5.4.** Ultra-Violet Spectrophotometry
  - **4.3.5.5.** Fourier Transform Infrared Spectroscopy
  - **4.3.5.6.** Gas Chromatography/Mass Spectrometry
  - **4.3.5.7.** Gas Chromatography-Infrared Spectroscopy
  - **4.3.5.8.** Polarimetry
  - **4.3.5.9.** Melting Point

4.4. Instruments:

- **4.4.1.** Ultraviolet light box
- **4.4.2.** Thin Layer Chromatography Development Tanks
- **4.4.3.** Ultraviolet Spectrophotometer (UV)
- **4.4.4.** Fourier Transform Infra-red Spectrometer (FTIR)
- **4.4.5.** Gas Chromatograph/Mass Spectrometer (GC/MS)
- **4.4.6.** Gas Chromatography-Infrared Spectroscopy (GC-IR)
- **4.4.7.** Polarimeter
- **4.4.8.** Melting Point Apparatus

4.5. Reagents/Materials: See Test Methods for analytical procedures (4.3.4)

4.6. Hazards/Safety: (See appropriate Test Methods)

4.7. Reference Materials/Controls/Calibration Checks:

4.7.1. Reference materials (See Reference Materials Test Method)
4.7.2. Blanks and Controls (See appropriate Test Method)
4.7.3. Calibrations/Verifications (See appropriate Test Method)

4.8. Procedures/Instructions:

4.8.1. Generally, a minimum of one preliminary and one confirmatory test is required to identify a substance. In some cases, additional testing may be necessary. GC-IR may be necessary for identification. (See 10.8.5)

4.8.2. Dry Powder Samples - Powder samples are examined by a series of tests which grow progressively more specific in identification. The tests which are employed can include a combination of Color Tests, Ultraviolet Spectrophotometry, Chromatography (thin-layer or gas), GC/MS, GC-IR, and/or Infrared Spectroscopy. Specialized testing techniques such as Melting point and Polarimetry are used with selected drugs to determine optical activity.

Since some components of drug samples can be masked or hidden during testing procedures, extraction procedures may need to be employed in an attempt to ensure that other substances are not being missed. Substances such as Acetaminophen, Ibuprofen, Aspirin, and Caffeine are commonly found in combination with controlled substances but the controlled substances may not be apparent using most screening methods.

4.8.3. Marked Tablets and Capsules - Tablets and capsules marked with pharmaceutical identifiers containing controlled substances are examined using reference identification and confirmation testing, at a minimum.

The Category B Pharmaceutical Identifier method is intended to be used only on tablets, capsules and pharmaceutical packaging consistent with that from a commercial manufacturer. When conducting an examination on a dosage form, care shall be taken to ascertain that the product has not been tampered with and is of legitimate, as opposed to clandestine, origin.

Markings that cannot be located in a published reference shall be treated as a general unknown and follow the drug examination procedures in 4.8.2.

4.8.3.1. Legend Drugs/Non-controlled Preparations - Those marked tablets and capsules that contain drugs that do not require a prescription and/or contain non-controlled prescription drugs shall not be examined. Reference identification is sufficient, unless there is evidence of tampering and/or reasons to suspect

tampering. These items can be re-submitted for analysis; however, confirmation may not be possible due to instrumental limitations and/or availability of a reference material. If a reference material is not available in the Drug Unit, the reference material shall not be purchased and an indication of the drug will be reported, if sufficient supporting data is present. The report shall also state a reason why the sample could not be confirmed.

4.8.4. Manufacturer sealed packaging: If the packaging of an over-the-counter and/or prescription drug is intact; lists the weight and/or dosage information and the contents on the package, a reference identification of the packaging may be used in lieu of analysis of the item. (See 4.10.8 and 4.11.8). A separate literature reference is not required.

4.8.5. Liquid Samples - Liquid samples are generally examined using the same techniques employed to examine powder samples. Adjustments may have to be made in the sample preparation and the procedures used. If a liquid sample has spilled out of its original container into an outer container (a plastic bottle containing a glass vial), the two liquids may be combined into one sample. This shall be documented in the notes.

4.8.5.1. Blood Contaminated Liquid Samples**:** Drug items that are suspected to be contaminated with blood may be screened by a biology analyst or may be withdrawn according to the Drug Unit's Physical Evidence Bulletin.

4.8.6. Clandestine Laboratory Samples - Samples from clandestine laboratory reaction mixtures require unique analysis and sampling procedures. Knowledge of procedures being utilized is important. Examination and identification of precursor compounds and finished product are necessary, as well as identification of intermediate products in some cases.

4.8.7. Plant Materials and Plant Material Preparations: - Plant materials are examined visually, macroscopically, and microscopically noting morphological characteristics. Additional tests such as Color Tests, Thin Layer Chromatography, Gas Chromatography, GC/MS, and GC-IR are available to be used to identify the components of plant materials, including Hashish, Hash oil, and residues.

4.8.7.1. Evidence with obvious or suspected powder or liquid added to vegetation will require examination by methods for dry powder and liquids to determine if additional drugs are present.

4.8.8. Psilocybic Mushrooms, Peyote Buttons, Opium Poppy, Khat, etc. – Mushrooms, peyote buttons, opium poppy samples, Khat and various

other materials are subjected to extraction procedures to remove the drugs of interest from the bulk of the sample prior to analytical testing. These extracts are then examined using routine procedures for dry powder or residue samples.

4.8.9. In all cases, comparison with a known reference material is required for a positive identification. The unknown sample and the reference material shall have been run on the same instrument using the same or similar methodology.

4.8.10. Disposal: Sample disposal by the analyst should be done within five working days from the completion of the analysis in order to prevent the accumulation and subsequent disposal of larger quantities of sample material. The analyst will maintain control of any sample waste until it is disposed. The Drug Unit Supervisor may direct the retention of samples for the use of training samples, proficiency samples, etc. This shall be documented in the case notes. At no time is the analytical waste to be allowed to accumulate without authorization.

Drug sample waste shall be disposed as per the Laboratory Drug Waste Management program. A secure location shall be selected in each laboratory for the purpose of collecting post-analysis drug waste such as tablets, capsules, powders, plant materials, etc. Liquid samples may require additional procedures (example: liquid PCP) for disposal.

GC/MS vials may be placed in the broken glass disposal boxes and disposed in the regular trash.

Drug Reference materials and bulk drugs have other requirements and restrictions. Refer to Test Method 31 for disposal.

Bulk drugs are subject to DEA disposal regulations. Refer to the Laboratory Waste Program and Drug Waste Management Program.

4.9. Records: Record in the examination documentation all notes, worksheets, data, sample preparation, detailed extraction procedures, reference identifications, and observations used to support the findings or results and opinions or conclusions. This would include:

4.9.1. All printouts of sample spectra generated for UV, GC/MS, FTIR, and GC-IR:

Additional sample and blank runs that are not used in comparison shall be retained in hardcopy form in the case file and/or stored electronically. If stored electronically, the data shall be retained on the instrument hard drive, or external hard drive. If data cannot be stored, a unit supervisor shall be contacted and all data shall be printed and included in the case

file. Data files shall not be over-written. Documentation of additional runs due to concentration, extractions and/or program changes shall be kept in the case notes. For GC/MS, if not stored electronically, at a minimum, the Total Ion Chromatogram (TIC) of the data that is not used for identification shall be printed and kept in the case file.

The reason for the additional data runs as well as the date shall be noted in the case file. The date on the data is sufficient if the data is included in the case file. If the data is not included, the date of the original run(s) needs to be documented in the case file.

4.9.2. **All other analytical tests:** If an observation, data, or calculation is rejected, the reason and date shall be recorded in the case file. The date the analytical test was performed is sufficient as the rejection date, unless the rejection was not made on the same date. If rejected data is printed and included in the case notes, the data shall be marked as rejected.

4.9.3. Standard spectra used for comparison to the unknown.

4.9.4. Photographs, if applicable.

4.9.5. Overlays or drawings of optical, physical or microscopic characteristics observed during the examination process. See 4.9.9 for Marijuana documentation.

4.9.6. Detail what tests were conducted on which items and/or sub-items.

4.9.7. The date of each test or observation shall be recorded in the case notes.

4.9.8. The solvents and extraction procedures used for analysis shall be documented on the Examination Worksheet.

4.9.9. Category A techniques shall have data that are reviewable. For Marijuana (Cannabis) a recording of detailed botanical characteristics observed is acceptable.

4.10. **Interpretations of Results:** For any method to be used for identification or confirmation, the test results must be considered “positive.” While “negative” test results provide useful information for ruling out the presence of a particular drug or drug class, these results have no value toward establishing the forensic identification of a drug.

4.10.1. All samples shall be compared with primary or secondary reference materials, which have been previously tested to verify their identity. (See Reference Materials Test Method)

All unknowns shall be evaluated prior to the comparison to a known.

4.10.2. Identifications: When a Category A technique is incorporated in an analytical scheme, then at least one other separate technique (from either Category A, B or C) shall be used for a positive identification. (For suspected Marijuana items, see Marijuana Test Method). This combination must identify the drug(s) present and must preclude a false positive identification.

4.10.3. Controlled or non-controlled substances can be conclusively identified when the results of the tests have been compared with a verified reference material of that substance and are of sufficient quality to permit identification.

4.10.4. The forensic scientist is not required to conclusively identify most non-controlled drugs, such as Caffeine, Ibuprofen, or Ampicillin.

4.10.5. Indications: In some instances the results of the examination will lack acceptable analytical results to positively conclude that a specific substance is present. This may be a result of the item not containing a sufficient amount of material or having a concentration that prevents a positive conclusion. Other possibilities include lack of an available reference material for comparison, and/or when an external library has been used (i.e. SWGDRUG or other library).

4.10.6. Inconclusive results: In some instances, examination yields no helpful or conclusive information that support neither identifications nor indications.

4.10.7. Reference Identifications: For some items, such as marked tablets of products that contain drugs that are not controlled, samples may not be removed for testing, but simply be visually examined for purposes of reporting what the item may contain based on the markings of the tablets, capsules, packaging, etc.

A reference identification is a valid secondary/presumptive test when analytical results are consistent with the reference information.

4.10.8. Reference Identification of manufacturer sealed packaging: If the packaging of an over-the-counter and/or prescription drug is intact; lists the weight and/or dosage information and the contents on the package, a reference identification of the packaging may be used in lieu of analysis of the item. A separate literature reference is not required.

4.10.9. Drug Preparations: There are occasions where a controlled substance is part of a preparation. The controlled substance shall be identified. The other active ingredients shall be indicated, at a minimum, when the

presence of the other ingredients may cause the controlled schedule to change. Analytical support of an indication is required.

4.10.10. Exempt Preparations: Exempted preparations that contain a controlled substance do not require a full examination, unless requested. See 4.11.9

4.11. Report Writing: Certificates of Analysis are generated by forensic scientists to report their results, opinions and interpretations following the examination of the item(s) of evidence listed on the report. The conclusions stated are a result of specifically what was tested and weighed (see Sampling 2.11). All analytical method(s) used in analysis shall be listed on the Certificate of Analysis for each item that was examined. The following are guidelines for reporting analytical results. It may be necessary to combine statements, make adjustments to accurately reflect analytical results and/or achieve consistency in reporting.

4.11.1. Analytical reports involving the examination of suspected controlled substances shall be written to offer information as to whether the materials examined are “controlled or non-controlled”.

In cases where an identification is made, the results shall be reported using the following or similar verbiage (see 2.11):

Item 001 was found to contain _____, a controlled substance.

or

Item 001 was found to contain _____, a non-controlled substance.

4.11.2. When a drug type is identified that is controlled federally but not a state controlled substance, the report shall include the drug identified as “a federally controlled substance”.

4.11.3. When an examination provides insufficient information to support an indication or identification, one or more of the following statements shall be used:

Item 001 – no controlled substance was identified (or similar wording).

or

Item 001 contained an insufficient amount of material (or concentration, or other reason) for identification.

4.11.4. In cases where an item is examined and a non-controlled substance is indicated, but not conclusively identified, the results shall be reported using the following or similar verbiage:

Item 001 – no controlled substance was identified.

and/or

Item 001 - Examination indicated the presence of _____, a non-controlled substance.

4.11.5. In cases where an item is examined and a controlled substance is not identified within that item, but is only indicated (due to insufficient material, concentration, or degradation, etc.), the results shall reflect the reason an identification could not be made using the following or similar verbiage:

Item 001 indicated the presence of _____, a controlled substance; however, there was insufficient material (or other reason) for complete identification.

or

Item 001 indicated the presence of _____, a controlled substance; however, this could not be confirmed due to insufficient material (or concentration of the sample, sample degradation, inconclusive testing results or other reason).

4.11.6. Reference Identifications without further testing: When reference identification is used and no other testing is performed, the report will reflect the item was visually examined and what the markings of the material indicate is present.

Reference Identifications shall be reported using the following verbiage:

Item 001 was visually examined. Reference(s) and markings indicated the presence of _____, a non-controlled substance. No confirmatory analysis was performed.

Also acceptable:
Item 001 was visually examined. Reference(s) and markings were consistent with a preparation containing _____, a non-controlled substance. No confirmatory analysis was performed.

4.11.7. Reference Identifications with examination**:** When reference identification is used and some analysis is performed, it may be necessary to combine or alter the approved report wording to accurately reflect this in a report.

For example: Item 001 – Reference(s) and preliminary examination indicated the presence of_____, a controlled/non-controlled substance.

4.11.8. Reference Identification of manufacturer sealed packaging: If a manufacturer sealed package is unopened, intact and the weight and contents are described in the labeling on the package, analysis of the items is not necessary. A reference identification of the packaging is sufficient. Additionally, the evidence description must reflect the "intact/sealed" packaging and basic details of the labeling (i.e. drug name(s) and dosage(s)).

For example: Item 001 – Reference identification of the sealed packaging indicated the presence of _____, a controlled/non-controlled substance. No confirmatory analysis of this item was performed.

4.11.9. Drug or Preparation Specific Results: There are occasions where the general result wording is insufficient to describe the test results accurately. In those instances, refer to the Test Method for the specific drug, or drug grouping.

Examples:

Item 001 was found to contain _____, a controlled substance and _____, a non-controlled substance. Reference(s) and examination were consistent with a preparation containing _____, a controlled substance.

4.11.10. Multiple drugs in one item: Many samples contain multiple substances and the results can be complex. The specified report wording may be adjusted to accurately describe the results of the examination. Multiple sentences should be used and run-on sentences should be avoided. It may be necessary to use several sentences.

4.11.11. Combining results: In a situation where there are two or more items with the same results, these may be combined to simplify the report. Weights may be reported as a total weight, as appropriate. However, it may be equally appropriate to list the individual weights. (See 3.11.3)

Example:
Items 001 and 002 were found to contain Cocaine, a controlled substance.
The total gross weight of items 001 and 002 was 2.50 grams.

4.11.12. Items not analyzed shall be reported as "not analyzed" and include a statement explaining why the item was not analyzed (e.g. unsuitable for analysis, insufficient material for analysis, etc.). This may not apply to

sub-items that are not examined or items that are administratively withdrawn.

4.11.13. If examinations by another forensic discipline are deemed appropriate, the contributor should be contacted and the following or similar statement shall be added to the report: "Item 001 was transferred to the Microanalysis (or other) Unit for analysis.

This will apply to those items (as specified in 10.8.5) that are transferred to the Indianapolis Laboratory for isomer determination or confirmation by GC-IR. In that event, one of the following statements should be used:

Item 001 was transferred to the Indianapolis Laboratory for further testing.

or

If the specific isomer needs to be determined, please re-submit the item(s) to the Indianapolis Laboratory for further analysis.

Example:

Item 001 was found to contain Fluoro-PB-22, a controlled substance. The specific isomer was not determined at this time.

If the specific isomer needs to be identified, please re-submit the item to the Indianapolis Laboratory for further analysis.

4.12. References:

4.12.1. Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations , 5th Ed., 2010-01-29, Part III (Methods of Analysis)

4.12.2. BNDD Analytical Manual: Analysis of Drugs (initial issuance), United States Department of Justice Bureau of Narcotics and Dangerous Drugs

4.12.3. Indiana State Police Laboratory Drug Waste Management Program in section 1 of the Laboratory Waste Management Program.

5. Color (Spot) Testing:

5.1. Scope: Color (Spot) Tests are preliminary tests (SWGDRUG Category C) used to indicate the presence or absence of certain drugs found in case samples. Spots tests have the advantage of being a quick, easy and inexpensive means to acquire information. A series of spot tests can be used to test an unknown sample in flow-chart fashion, leading to two or three possible substances out of hundreds. This Test Method is intended to provide instruction for the proper use and interpretation of Color (Spot) Tests.

5.2. Precautions/Limitations:

5.2.1. The Drug Unit Reagent Preparation Guide contains a list of commonly used and acceptable color test reagents. This list is not necessarily all-inclusive. Color Tests not listed or contained in the Reagent Preparation Guide are acceptable if properly validated (See Laboratory Method Validations policy).

5.2.2. Color Tests are suitable for use on powders, liquids, residues, tablets, and capsules. It may be necessary to make minor adaptations to perform these types of tests on liquid or plant material samples.

5.2.3. The tests are generally destructive and the sample cannot be used further in analysis.

5.2.4. These tests are non-specific and therefore cannot provide positive identification of a particular substance. In most situations, the color reaction produced is not confined to a single compound, but rather a number of related compounds in a particular class of substances (i.e. drugs with similar structures may give the same reaction). These tests can aid in narrowing the possibilities by the process of elimination.

5.2.5. Not all Color Tests (or Spot Tests) produce a color, but rather a characteristic reaction.

5.2.6. The reactions of several tests correlate with particular functional groups or other drug structures. There are reactions that are observed that are not fully understood, but have in practice been repeatable and reliable indicators of a particular substance or group of substances.

5.2.7. False positives and false negatives are possible.

5.2.8. Color Test reactions can be influenced by the concentration of the controlled substance and by interferences from diluents or other substances. It is possible to observe a combination of colors produced by the reaction.

5.2.9. It is possible to have secondary color reactions over time, however decomposition occurs rapidly. Observations should be made in a timely manner to avoid misinterpretation.

5.2.10. Forensic Scientists must possess the visual ability to distinguish color and detect slight color changes for proper documentation and evaluation of color test reactions.

5.2.11. A series of color tests is most appropriate for use as a method for screening samples. If the sample is a residue, a single test may be appropriate.

5.3. Related Information:

5.3.1. Appendix 1 – Forms and Worksheets
5.3.2. Appendix 2 – Abbreviations
5.3.3. Appendix 3 – Definitions
5.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
5.3.5. Other Test Methods
- **5.3.5.1.** General Drug Analysis
- **5.3.5.2.** Reference Materials Test Method
- **5.3.5.3.** Drug Unit Training Manual – Spot Test module

5.4. Instruments:

5.4.1. Fume Hoods/fume absorbers

5.5. Reagents/Materials:

5.5.1. Reagents: (See Reagent Preparation Manual)
5.5.2. Organic Solvents (methanol, petroleum ether, chloroform, etc.)
5.5.3. Ceramic Well plate
5.5.4. Disposable test tubes
5.5.5. Evaporating dish
5.5.6. Spatulas, pipettes

5.6. Hazards/Safety:

5.6.1. Routine use of concentrated acids and bases
5.6.2. Fumes (exposure and inhalation hazards)
5.6.3. Carcinogens
5.6.4. Poisons
5.6.5. Reproductive Hazards

5.7. Reference Materials/Controls/Calibration Checks:

5.7.1. Reagents shall be verified with a known reference material when the solution is made and on a monthly basis at a minimum. See Reagent Preparation Guide for specifics. (See also 5.9.3)

5.7.2. Infrequently used Spot Test reagents shall be verified with a reference material at the time of use. (see 5.9.2)

5.7.3. Negative Controls (Blanks) shall be run in conjunction with multi-step spot tests to demonstrate that the combination of reagents is blank or does not produce a color reaction. (5.9.4)

5.7.4. If a color or spot test fails the verification process, it must be discarded. The reagent shall be re-made and verified.

5.8. Procedures/Instructions: Tests can be performed directly on a portion of the sample or extract in a small test tube, spot plate or evaporating dish.

5.8.1. Place a small amount of sample, positive control or negative control in a well of a clean, dry ceramic spot well plate or test tube.

5.8.2. Add 2-3 drops of the desired reagent to the well.

5.8.3. Observe and record reactions on the Examination Worksheet.

5.8.4. If one color test is used, then the selected test shall be relevant to the compound being identified and produce a positive result.

5.9. Records: Any reactions, including gas evolved, color changes, and/or precipitate formed, shall be recorded on the Examination Worksheet with the date the test was performed. A lack of reaction (e.g. no color reaction (NCR), no reaction (NR), etc.) shall also be recorded in the analysis notes.

5.9.1. Ten basic spectral colors are recommended to describe reactions. Variations in color are indicated by combining two colors (e.g. red-brown), with the second color being the dominant color.

5.9.2. Reference materials and blanks used to verify infrequently used reagents shall be recorded in the notes or on the Reagent Preparation and Verification log.

5.9.3. Reagent preparation and verifications shall be recorded on the Reagent Preparation and Verification log and shall include the lot numbers of the chemicals used, color reaction observed, date, initials of the preparer/verifier and the name, source and lot numbers of the substance(s) used to verify the reagents. The reagent bottle shall be labeled with the date of preparation and initials of the preparer. (See 5.7.1)

5.9.4. Results of the negative controls/blanks used for multi-step reagents shall be documented in the case notes.

5.10. Interpretations of Results: Color interpretations are subject to the opinions of the forensic scientist performing the test. Spot tests can give a characteristic reaction if a particular substance is present. Others can give several different reactions according to which substance is present and can be used to help distinguish between different classes of drugs, depending on which color forms.

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5.10.1. A positive result will be based upon an initial expected reaction and/or the color progression of the reaction. (See 5.12.1)

5.10.2. A positive result does not indicate that a specific drug is present. It indicates that a certain class of drug may be present.

5.10.3. A negative test, or no reaction, indicates the absence of a substance or an insufficient amount of material.

5.11. Report Writing: N/A

5.12. References:

5.12.1. Analysis of Drugs and Poisons 3rd Edition, Clarke, E.G.C, London, Pharmaceutical Press, 2004.

5.12.2. Isolation and Identification of Drugs, Clarke, E.G.C, London, Pharmaceutical Press, 1986.

5.12.3. Spot Tests in Organic Analysis 7th Edition, Feigl, F, New York: Elsevier Scientific Publishing Company, 1966.

5.12.4. Forensic Science Handbook Volume II, Saferstein, R, Englewood Cliffs, NJ: Prentice Hall, 1988.

5.12.5. Tannic Acid as a Field Test for Caffeine, Hueske, EE, Microgram, Vol. XV, No. 9, September, 1982, p. 158.

5.12.6. The Weber Test: A Color Test for the Presence of Psilocin in Mushrooms, Garrett, A.S., Clemens, S.R., Gaskill, J.H.SWAFS Journal, Vol. 15, No. 1, April, 1993, pp.44-45.

5.12.7. United States Department of Justice Drug Enforcement Administration, Analysis of Drugs Manual, 2nd Ed., February, 1999.

5.12.8. A New Field Test Reagent, Ferris Van Sickle, Laboratory Notes, June 4, 1974.

5.12.9. Chemical Field Tests for Narcotics and Dangerous Drugs, US Department of Justice, Bureau of Narcotics and Dangerous Drugs.

5.12.10. Color Test to Differentiate Between Cocaine and Lidocaine, Carolyn Ruybal

5.12.11. The Multiple Testing of Suspected Drugs to Minimize False Positives, Robert B. Carroll, Ph. D.

5.12.12. Color Tests-Methcathinone/Methamphetamine, Terry Dal Cason

5.12.13. Screening Test for Amphetamine, Fleischer, David (NYC Police Department, New York, NY), Microgram, Vol. VIII, No. 8 (August, 1975).

6. Ultraviolet Spectrophotometry:

6.1. Scope: Ultraviolet (UV) Spectrophotometry is a SWGDRUG Category C method of analysis that is widely used as a screening test in forensic drug analysis. In combination with other analytical data, this technique provides supporting data for identification of controlled substances. Under carefully controlled conditions, Ultraviolet Spectrophotometry can be used as a method of quantitation for cases involving product tampering. This Test Method is intended to give guidance and instruction for proper use and interpretation of data generated from the UV instrument.

6.2. Precautions/Limitations:

6.2.1. The UV gives limited structural information and some selectivity to allow for some distinction between similar substances. However, it does not give specific results and cannot be used as a conclusive method of identification.

6.2.2. Not all solvents are suitable for use in UV. Solvents should be selected that do not absorb in the UV region.

6.2.3. Quartz cuvettes should be used for UV analysis. Glass and plastic cuvettes may not be suitable for analysis in the UV range.

6.2.4. Compounds that lack suitable chromophores provide no absorbance pattern.

6.2.5. Different compounds may have very different absorption maxima depending on the solvent used and the solubility of the sample.

6.2.6. Highly concentrated samples and intensely absorbing compounds may shift the absorbance maxima and/or saturate the spectrum and therefore must be examined in dilute solution.

6.2.7. Strong UV absorbing substances can mask the presence of other weaker UV absorbing substances. Additional testing, and/or extraction, is necessary to reveal weaker UV absorbing substances.

6.2.8. The presence of interfering substances can influence the absorption spectrum by shifting the maxima.

6.2.9. Solvent polarity and pH can affect the absorption spectrum of an organic compound.

6.2.10. Chemical composition may change during analysis.

6.2.11. It is possible to recover the sample, if necessary.

6.3. Related Information:

6.3.1. Appendix 1 – Forms and Worksheets
6.3.2. Appendix 2 – Abbreviations
6.3.3. Appendix 3 – Definitions
6.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
6.3.5. Other Test Methods
6.3.5.1. Reference Materials
6.3.5.2. General Drug Identification
6.3.5.3. Drug Unit Training Manual – UV module

6.4. Instruments: Ultraviolet Spectrophotometer capable of recording spectra in the UV range generally from 400 – 200 nanometers.

6.5. Reagents/Materials:

6.5.1. 0.5N Sulfuric Acid (H_2SO_4) (UV cutoff point 190nm)
6.5.2. 0.45N Sodium Hydroxide (NaOH) (UV cutoff point 225nm)
6.5.3. Methanol (MeOH) (UV cutoff point 210nm)
6.5.4. Chloroform ($CHCl_3$) (UV cutoff 245nm)
6.5.5. Water (UV cutoff 205nm)
6.5.6. Quartz Cuvette (UV cutoff point 170 nm)

6.6. Hazards/Safety:

6.6.1. General Drug Exposure
6.6.2. Dilute acids and bases
6.6.3. Organic solvents

6.7. Reference Materials/Controls/Calibration Checks:

6.7.1. Reagents: Reagent acids and bases used in UV analysis shall be verified by checking the pH of the solution at the time it is prepared.

6.7.1.1. If the reagent fails verification process, then it must be discarded and the reagent shall be re-made and verified.

6.7.2. Performance Checks: The UV Instrument shall be performance checked once per month using a Holmium Oxide reference material. The resulting absorbance maxima shall be within +/- 2 nanometers of the expected value to be considered satisfactory.(See 6.9.2 and 6.9.3)

6.7.2.1. In the event that performance checks are found to be unsatisfactory, the instrument shall be taken out-of-service and steps taken to restore the instrument to proper working order.

6.7.3. Any instrument that is out-of-service shall be visibly marked.

6.7.4. When an instrument is taken out-of-service for maintenance and/or repair, performance checks shall be performed prior to resuming casework on that instrument.

6.7.5. An infrequently used instrument may be placed in an "Inactive" status and the normal performance verification procedures may be suspended. Normal performance verification procedures shall be resumed prior to use in casework analysis.

6.7.6. Reference material spectra that have been generated in-house (prior to 2006), do not contain lot numbers, and have been included in the case notes need to document the source of the reference spectra (e.g. "from FWRL UV library").

6.8. Procedures/Instructions:

6.8.1. Samples are routinely examined in 0.5N Sulfuric Acid, methanol (MeOH) or 0.45N Sodium Hydroxide.

6.8.2. Samples may be run as received or extracted. Some samples, such as mushrooms, suspected LSD gelatin squares, etc. require extraction prior to UV analysis.

6.8.3. Generally the wavelength range scanned is approximately 400nm to 200nm, depending on the UV cut-off of the solvent being used. (See 6.5)

6.8.4. Run a solvent blank, or background, using the same solvent that will be used to measure the sample. A solvent blank or background shall be run before each sample.

6.8.5. A small amount of sample is placed in a cuvette and dissolved in the appropriate solvent. (See 6.2.3)

6.8.6. Obtain the UV absorption spectrum by scanning the sample and solvent matrix. (See 6.8.3)

6.8.7. Print the UV spectrum. (See 6.9.4)

6.8.8. Samples can be recovered and additional tests performed, if necessary.

6.8.9. If the UV absorbance pattern and maxima indicate the presence of a non-controlled substance such as Acetaminophen, Caffeine, Ibuprofen, or Aspirin, the sample shall be extracted and analyzed via Gas Chromatography/Mass Spectrometry, Gas Chromatography-Infrared Spectroscopy, or Fourier Transform Infrared Spectroscopy. However, if

the sample is analyzed unextracted using a confirmatory technique, and a controlled substance is present, the sample does not have to be extracted.

6.8.10. Preventative maintenance: The UV instrument has no routine maintenance. In the event of a source failure or malfunction, it shall be replaced. If the instrument fails its performance checks, it shall be taken out of service and repaired.

6.9. Records:

6.9.1. Reagent acid and base preparation and verification shall be documented on the Reagent Preparation and Verification log. The bottle shall bear the date of preparation and the initials of the preparer.

Maintenance: Each UV instrument shall have a maintenance log.

6.9.2. Calibration/verification information shall be documented in the Calibration Verification Log.

6.9.3. The status of any inoperable, in-active or out-of-service instruments shall be reflected in the maintenance log.

6.9.4. All UV spectra printouts shall contain information regarding the observed maxima absorbance and the solvent used.

6.9.5. Result of the solvent blank used for UV analysis shall be noted on the Examination Worksheet. The solvent blank shall be printed or saved.

6.9.6. The laboratory case number, item number, and date of the examination shall appear on the printout and may be computer generated. The forensic scientist shall initial the data by hand.

6.9.7. All maxima used for identification or indication shall be marked and summarized on the Examination Worksheet, including unit of measure (nm).

6.9.8. References used for comparison of uncommon substances should be included and/or the source of the reference specifically documented on the Examination Worksheet, if used for identification or indication. It is not necessary to include a reference included for routinely encountered substances (e.g. Cocaine, Methamphetamine, Acetaminophen, etc.) that have characteristic absorption patterns that are easily recognizable.

6.9.9. In instances where a sample is run multiple times, the existence of multiple runs shall be documented on the Examination Worksheet and the data shall specify which run has been included (e.g. Run 1, Run 2). The data

from each run shall be labeled with a Run number. The reason for multiple runs shall also be documented. If the UV is not capable of saving data, all runs shall be printed and saved in the case record.

6.10. Interpretations of Results: UV absorption patterns can be indicative of a substance or more commonly, a particular class of substances. The forensic scientist should be familiar with the common spectra observed in the laboratory and reference listings of standard UV absorbance maxima.

6.10.1. The absorbance spectra of unknowns are compared with known reference material spectra and/or with tables of standard UV maxima using a +/- 2 nm uncertainty window to narrow the list of possible compounds, if applicable.

6.10.1.1. If the peak is outside the +/- 2 nm window, additional presumptive testing shall be performed.

6.10.2. The shape, position and intensity of the absorbance maxima shall be evaluated when making determinations.

6.10.3. It is recognized that mixtures result in absorbance patterns that are influenced by components with various absorptivities and concentrations. This may result in absorbance shifts or blending of absorbance maxima.

6.11. Report Writing: N/A

6.12. References:

6.12.1. Analysis of Drugs and Poisons 3rd Edition, Clarke, E.G.C, London, Pharmaceutical Press, 2004.

6.12.2. Isolation and Identification of Drugs, Clarke, E.G.C, London, Pharmaceutical Press, 1986.

6.12.3. Drug Unit's Reagent Preparation Guide for Composition of Color Test Reagents, Spray Reagents, and Acid/Base Reagents

6.12.4. Resource Manual on Quantitation

6.12.5. Principles of Instrumental Analysis. 6th ed. Skoog, et al Thomson Brooks/Cole. 2007, 169-173.

6.12.6. Instrumental Data for Drug Analysis, Mills III, Terry, and Roberson, J. Conrad. 2nd Ed. New York, New York: Elsevier Science Publishing Company, 1987.

6.12.7. United States Department of Justice Drug Enforcement Administration, Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations, 2nd Edition, Supplemental Document SD-2, 01/29/2010.

7. Thin Layer Chromatography:

7.1. Scope: Thin Layer Chromatography (TLC) is a SWGDRUG Category B technique used for separation and tentative identification of controlled substances. This Test Method is intended to provide instruction for the proper use and interpretation of Thin Layer Chromatography data.

7.2. Precautions/Limitations: Thin Layer Chromatography has a number of analytical advantages. It is a relatively rapid, cost-effective method of analysis. It enables many samples to be screened simultaneously against multiple known reference materials. Samples can be recovered if non-destructive visualization techniques are used.

7.2.1. Analytes of interest should be stable in the solvent system being used.

7.2.2. TLC has a lower sensitivity and resolution than other chromatographic methods, such as gas-chromatography. A sufficient amount of material must be available to perform this test properly. If a comparison is made, the amounts of sample and reference material should be similar.

7.2.3. Most substances dissolve readily in methanol (MeOH) and can be applied (spotted) along the origin of the TLC plate. It may be necessary to dissolve some substances in a more appropriate solvent to ensure a more concentrated sample is available for the test.

7.2.4. TLC plates are of a finite size and so it is not possible to use this method to separate the multitudes of substances in existence and provide a conclusive means of identification.

7.2.5. Irregularities in the TLC plate thickness can have an effect on separation and prevent quality conclusions from being made.

7.2.6. Salt forms may have an effect on separation, spot shape and Rf values. Some salt forms produce tailing or streaking spots.

7.2.7. Chemicals used in the solvent systems must be analytical grade equivalent, or better, and be mixed thoroughly to achieve sufficient separation of drugs in the same class.

7.2.8. Loss or evaporation of solvent can delay or skew the separation of substances. The TLC chambers should be tightly sealed to prevent solvent loss.

7.2.9. Loss of volatile samples can occur by heating the TLC plates (i.e. Methamphetamine in basic TLC systems).

7.2.10. Contamination of solvent systems can have an effect on separation by altering the polarities of the solvent system.

7.3. Related Information:

7.3.1. Appendix 1 – Forms and Worksheets
7.3.2. Appendix 2 – Abbreviations
7.3.3. Appendix 3 – Definitions
7.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
7.3.5. Other Test Methods
7.3.5.1. Reference Materials Test Method
7.3.5.2. General Drug Identification

7.4. Instruments: Long and short wave UV light box

7.5. Reagents/Materials:

7.5.1. TLC development chambers- generally rectangular glass chamber with a lid.

7.5.2. Stationary Phase - TLC plates. Silica Gel (250 µm) coated glass plates are most commonly used. Aluminum backed silica coated plates are also acceptable.

7.5.2.1. The use of fluorescent indicators is recommended.

7.5.3. Mobile Phase – solvent systems depend on the compounds to be separated and stationary phase used. Systems used should be stable in air or when mixed with acids and bases. It should be easily removed from the plate after development and should not react with the substances to be separated. (See Reagent Preparation Guide)

7.5.4. Capillary tubes or micropipettes.

7.5.5. Reference Materials (as appropriate for the particular drug or drug class).

7.5.6. Reagent over-sprays/visualization reagents (See Reagent Preparation Manual).

7.5.7. Standard 12 inch ruler for approximate Rf calculation, if applicable.

7.5.8. Pencil for marking spots.

7.6. Hazards/Safety: Hazardous Chemical Exposure, including potential carcinogen exposure.

7.6.1. Mobile phases and visualizing reagents should be prepared in the hood.

7.6.2. Any spraying of visualization reagents, or over-sprays, shall be performed with the hood on and the spray directed into a spray box.

7.6.3. TLC plates present a chemical exposure hazard after development. Plates should be viewed in a timely manner and disposed in a glass disposal box.

7.6.4. Physical Hazards: broken glass potential.

7.7. Reference Materials/Controls/Calibration Checks:

7.7.1. Primary or secondary reference materials and a solvent blank shall be used simultaneously with unknowns in all cases.

7.7.2. In the event that spots appear in the blank(s), the TLC examination is invalid and the blank shall be re-run under the same conditions. If the second blank continues to be unacceptable, steps to locate and remove the source of contamination shall be taken prior to any further TLC analysis. This may necessitate re-sampling of evidence.

7.7.3. Once the source of contamination has been eliminated, the entire test including unknowns, reference materials and blanks shall be re-run.

7.8. Procedures/Instructions:

7.8.1. Various solvent systems will be utilized depending on the material to be tested. In all cases a minimum of one solvent system shall be used. Two solvent systems are recommended for greater selectivity.

7.8.2. Tank Preparation

7.8.2.1. Each forensic scientist is responsible for ensuring the quality, and freshness of the solvent systems. It will be their decision to determine if it is suitable for use or if a fresh mixture is appropriate. That person shall discard the solvent and make a “fresh” system.

7.8.2.2. Single component solvent systems shall be made up and discarded on an as needed basis.

7.8.2.3. Multi-component solvent systems have a short shelf life and should be freshly mixed just before using or daily. These systems should be changed after 2-3 full plates (20cm X 20cm) or the equivalent, or daily, whichever comes first.

7.8.2.4. The analyst preparing to run multi-component TLC systems shall evaluate the existing solvent system, if any, discard and prepare the new solvent mixture if necessary. The date of this fresh mixture is to be recorded on a tag/index card/note/etc. affixed to the tank noting the tank has been prepared.

7.8.2.5. After the solvent system is mixed, add it to the tank. The developing solvent should be approximately 0.5 cm deep. Allow to equilibrate in the developing chamber.

7.8.3. Plate Preparation

7.8.3.1. Unknowns and reference materials are routinely dissolved in Methanol, Chloroform or Petroleum Ether.

7.8.3.2. Spot the sample, reference materials and solvent blanks approximately 1 cm up from the bottom edge of a dry thin layer chromatography plate.

7.8.3.3. Reference materials shall be run simultaneously with unknown samples on the same plate for comparison. The concentration of the sample and the reference material should be approximately the same.

7.8.4. Place the plate in the tank, sealing the lid tightly. The plates should be allowed to develop approximately 10 to 20 centimeters or to the top of the plate.

7.8.5. After completion of the development, remove the TLC plates from the tank and allow them to dry. It is permissible to use a dryer to expedite the drying process.

7.8.6. View the dried plates under long (360nm) and/or short (254nm) wavelength UV light.

7.8.7. Where appropriate, mark the spots viewed under short and/or long wave UV lightly in pencil prior to proceeding with chemical visualization reagents.

7.8.8. Spray the TLC plate with appropriate visualization or color developing reagents (e.g. – Fast Blue BB, Iodoplatinate, Potassium Permanganate, p-DMAB, Ninhydrin, etc.), and mark for identification purposes, if appropriate.

7.8.9. Rf values can be calculated, if necessary or desired.

7.8.10. Preparative Thin Layer Chromatography: When samples contain other organic substances that interfere with analysis, this method can be used to clean up or remove those substances for other testing such as FTIR or GC/MS.

7.8.10.1. A neutral solvent system should be used to avoid altering the original salt form of the analyte.

7.8.10.2. Prepare TLC plates as per 7.8.3, and follow procedures in 7.8.4 - 7.8.7.

7.8.10.3. When plate is dry, scrape off the desired area and wash thoroughly with solvent (methanol) in a beaker. Filter to remove the silica gel.

7.8.10.3.1. It may be necessary to use extraction procedures from an aqueous acidic or basic solution to separate the substance from the silica gel.

7.9. Records:

7.9.1. Solvent system(s) and method(s) of visualization used shall be documented on the Examination Worksheet.

7.9.2. Conclusions as to the solvent blank and all spots in the unknown sample shall be recorded in the notes. A check box is sufficient to document an acceptable blank.

7.9.3. The source and lot numbers of reference materials used for tentative identifications/indications shall be in the case notes. An attached sheet may be appropriate.

7.9.4. It is recommended, but not required, to list all reference materials used in the TLC comparison.

7.9.5. If no spots are visualized in the unknown, the use of at least one reference material shall be documented.

7.9.6. Photographs may be taken of TLC plates, if desired. Under no circumstances are TLC plates to be placed directly on a photocopier and photocopied. (See 7.6.3)

7.10. Interpretations of Results:

7.10.1. Positive indication of the unknown sample will be based on comparable color and location of the sample spot(s) on the plate relative to the reference material.

7.10.2. A positive result recorded refers to the drug reference material that was used in the comparison.

7.11. Report Writing: N/A

7.12. References:

7.12.1. Clarke's Isolation and Identification of Drugs. 2nd Edition; Clarke, E. G.C., The Pharmaceutical Press, 1986.

7.12.2. Clarke's Analysis of Drugs and Poisons. 3rd Edition; Clarke, E. G.C., The Pharmaceutical Press, 2004

7.12.3. United States Department of Justice Drug Enforcement Administration, Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations, 2nd Edition, Supplemental Document SD-2, 01/29/2010.

7.12.4. Instrumental Applications in Forensic Drug Chemistry – Proceedings of the International Symposium; USDOJ Office of Science and Technology, May 29-30, 1978.

8. Fourier Transform Infrared Spectroscopy:

8.1. Scope: Fourier Transform Infrared Spectroscopy (FTIR) is a SWGDRUG Category A method of analysis. This method uses reflected, absorbed or transmitted radiant energy in the mid-infrared region of the electromagnetic spectrum to produce data capable of providing specific chemical and structural information of a substance. It is particularly useful in determining salt forms of controlled substances, differences between closely related compounds and identification of small or low molecular weight compounds. This Test Method is intended to give guidance for proper use and interpretation of FTIR data.

8.2. Precautions/Limitations:

8.2.1. FTIR can be used as a confirmatory technique when the substance being analyzed is in a relatively pure form or when the sample is mixed with substances that do not absorb in the mid-IR region. Most casework samples are not pure enough to permit identification as received. It is usually necessary to perform extraction procedures, which can result in a substantial loss of sample.

During separation procedures it is possible that chemical changes in the material (i.e. salt form conversions) may occur.

8.2.2. FTIR requires a larger sample than most other techniques to perform the test. However, the sample is usually recoverable and could be used for other testing procedures.

8.2.3. Optical isomers cannot be distinguished using this method of analysis.

8.2.4. Gas Samples - Gas phase spectra differ from condensed-phase spectra because the molecules are free to rotate in a gas which minimizes intermolecular interaction. This results in more fine structure and fewer peaks.

Samples that must be analyzed as a gas must be stable in that form at room temperature. An air tight gas cell must be used for this type of analysis.

8.2.5. Pure samples may give different spectra due to polymorphism.

8.2.6. Environmental conditions, such as high humidity, can complicate the spectrum by adding additional peaks (typically CO_2 and H_2O).

8.2.7. Attenuated Total Reflectance (ATR) spectra are similar to transmission spectra; however the absorbance bands are shifted.

8.3. Related Information:

8.3.1. Appendix 1 – Forms and Worksheets
8.3.2. Appendix 2 – Abbreviations
8.3.3. Appendix 3 – Definitions
8.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
8.3.5. Other Test Methods
8.3.5.1. General Drug Analysis
8.3.5.2. Reference Materials

8.4. Instruments:

8.4.1. Fourier Transform Infrared Spectrometers (FTIR) of various makes and models capable of recording spectra in the mid-IR range of approximately 4000-450 cm^{-1}.
8.4.2. Attenuated Total Reflectance Apparatus (ATR)
8.4.3. Diffuse Reflectance Apparatus
8.4.4. Hydraulic press and Pellet dies
8.4.5. Desiccators with desiccants
8.4.6. Mechanical shaker/grinder/mixer

8.5. Reagents/Materials:

8.5.1. Methanol
8.5.2. Polystyrene Reference Material and/or other known Reference Materials.
8.5.3. Infrared Grade Potassium Bromide (KBr)
8.5.4. Salt plates Sodium Chloride (NaCl) or Potassium Bromide (KBr)
8.5.5. Gas cell and syringe
8.5.6. Mechanical shaker vials and caps
8.5.7. Solvent cover
8.5.8. Glass mixing beads

8.6. Hazards/Safety:

8.6.1. Exposure to chemicals and drugs during analysis
8.6.1.1. MeOH
8.6.1.2. Acetone
8.6.1.3. $CHCl_3$
8.6.1.4. Drugs – See individual MSDS for specifics

8.6.2. Maintenance Hazards: IR light/ radiation exposure. Optical hazards due to laser exposure.

8.7. Reference Materials/Controls/Calibration Checks:

8.7.1. Calibration and/or performance checks shall be run weekly using a known reference material and documented in the maintenance log. (See 8.9.2)

8.7.2. Instruments configured for transmission mode shall be verified each week using a polystyrene reference material and a background or air blank.

8.7.3. Instruments configured for ATR mode shall be verified each week using a known Cocaine reference material and a background or air blank.

8.7.4. Performance checks: The most intense peak in each of three regions (4000-2000 cm^{-1}, 2000-1200 cm^{-1}, and 1200-450 cm^{-1}) shall be within +/- 4 cm^{-1} of the expected value.

8.7.5. In the event that calibration or performance checks are found to be unsatisfactory, the instrument shall be taken out-of-service and measures taken to restore the instrument to proper working order.

8.7.6. Any instrument that is out-of-service shall be visibly marked and the maintenance log shall reflect the inoperable status. (See 8.9.3)

8.7.7. When an instrument is taken out-of-service for maintenance and/or repair, performance and/or calibration checks shall be performed prior to resuming casework on that instrument.

8.7.8. Performance check procedure:

8.7.8.1. Run a background spectrum.
8.7.8.2. Optional: run an air blank.
8.7.8.3. Scan the reference material and print the spectrum.
8.7.8.4. Record and store the performance verification spectra in the instrument calibration and maintenance log.

8.7.9. If unexpected peaks appear in the background or air blank, steps shall be taken to restore the instrument to proper operating status (e.g. the ATR crystal and anvil shall be cleaned, etc.) and the background or air blank shall be repeated.

8.7.10. If the background or air blank continues to be unacceptable, steps shall be taken to resolve the issue prior to further analysis.

8.8. Procedures/Instructions:

8.8.1. All instruments shall be operated according to their respective operations manuals.

8.8.2. Solid Samples: Solid samples, including powders as received, should be analyzed using the ATR accessory, KBr Pellets, Diffuse Reflectance or FTIR microscope.

8.8.3. Liquid Samples: Examination of liquid samples may be accomplished by use of a liquid cell, a headspace sample in a gas cell, liquid between salt

plates (NaCl or KBr) or examination by using Diffuse Reflectance or ATR. It may be necessary to use a solvent cap to prevent loss by evaporation.

8.8.4. Gas Samples: Gas or Headspace samples can be examined by using a gas cell. Clean the gas cell by evacuating it with an air stream. Seal the cell, and scan the background. Introduce the sample into the cell and scan the sample.

8.8.5. KBr pellets: Mix approximately 1-2 mg of the sample with approximately 100 mg of Potassium Bromide and mix thoroughly. Place the mixture in a pellet die and press using a hydraulic laboratory press. The directions for the operation of the pellet press should be consulted. IR-grade potassium bromide shall be used for all discs.

8.8.6. Salt plates: Run a background spectrum of the salt plate(s) being used. Spread a solution of the sample (or neat sample) onto a salt plate in a thin layer. Let the solvent evaporate and, if necessary, place a second salt plate of like material on top of the first plate, creating a "sandwich". The sample is then placed in the sample holder and the spectrum is scanned.

8.8.7. When Diffuse Reflectance or Attenuated Total Reflectance is used, it is possible that little or no sample preparation is required.

8.8.8. The surfaces of accessories shall be cleaned with methanol or acetone prior to the collection of each sample and when sample acquisition is complete.

8.8.9. A background or an air blank shall be run before the collection of each sample. (See 8.7.9 – 8.7.10)

8.8.10. Drugs are examined using a wavelength range of approximately 4000 cm-1 to 450 cm^{-1}. The wavelength range used with an ATR accessory shall be approximately 4000 cm^{-1} to 650 cm^{-1}, depending on the crystal composition.

8.8.11. Samples and reference material spectra should be evaluated in %Transmittance units.

8.8.12. Sample Analysis Procedure:

8.8.12.1. Run and print a background spectrum. (See 8.7.9 – 8.7.10)
Place the sample on/in the accessory or in the sample holder.
Run and print the unknown sample spectrum.
Clean the accessory, if applicable.
Repeat with next sample.

or

8.8.12.2 Run a background spectrum. (See 8.7.9 – 8.7.10)
Run and print an air blank.
Place the sample on/in the accessory or in the sample holder.
Run and print the unknown sample spectrum.
Clean the accessory, if applicable.
Repeat air blank and proceed with next sample.

8.8.13. Maintenance: the desiccant shall be changed as necessary. Source and laser replacement shall be replaced on an as needed basis. Operations such as mirror alignment shall be performed as necessary to keep the instrument in optimal working order.

8.9 Records:

8.9.1 The FTIR instrument used in analysis shall have a unique identifier and be documented on the Examination Worksheet.

8.9.2 Maintenance: Each FTIR instrument shall have a maintenance log.

8.9.3 The status of any instrument that is out of service shall be recorded in the maintenance log as “out of service”. The return to service shall be recorded after satisfactory performance and/or calibration checks have been performed.

8.9.4 All FTIR spectral data shall be labeled with the case and item numbers, the date, and initials of the examiner.

8.9.5 In instances where a sample is run multiple times, the existence of multiple runs shall be documented on the Examination Worksheet and the data shall specify which run has been included (Run 1, Run 2). The data from each run shall be labeled with a Run number. The reason for multiple runs shall also be documented. If the FTIR is not capable of saving data, all runs shall be printed and saved in the case record.

8.9.6 An acceptable background or air blank shall be noted on the Examination Worksheet.

8.9.7 The nature of the sample shall be recorded, e.g. KBr pellet, solution, gas cell, ATR or Diffuse reflectance, KBr or NaCl salt plate, etc. on the spectral data and on the Examination Worksheet.

8.9.8 If performed, extraction procedures shall be noted on either the infrared spectrum or the Examination Worksheet. It is sufficient to label the spectrum as “extracted”, if the details of that extraction are included on the Examination Worksheet and vice versa.

8.9.9 For identification, all spectral data shall be compared to a known reference material and/or a user generated spectral library that has been generated on that instrument.

8.9.10 The source and lot numbers of reference materials used for comparison shall be included in the case file.

8.9.11 All data generated from FTIR analysis, including blanks (backgrounds or air blanks) and reference material spectra used for comparison, shall be printed, labeled appropriately, and documented in the case file.

8.9.12 When literature references or reference spectral data are used in analysis, the source of the spectral data shall be documented in the case file, when appropriate.

8.9.13 The conclusion or results from the analysis of the infrared spectrum shall be documented on the Examination Worksheet as positive or similar verbiage, or indication of a specific drug, including the salt or base form, if relevant.

8.9.14 In cases where neither identification, nor a sufficient indication, can be made based on the spectral data, the results shall be labeled with an evaluation such as unidentified or other similar verbiage.

8.9.15 All reference material spectra shall be maintained electronically on the instrument that generated it, at a minimum. This data shall be backed up either in hard copy form, on an external hard drive or other storage medium.

8.10 Interpretations of Results:

8.10.1 The spectrum must be well resolved and of a sufficient intensity.

8.10.2 Identifications shall be made by direct comparison to a known reference material of the substance being analyzed, and/or an entry from a user generated spectral library, generated on the same instrument.

8.10.3 The comparison can be accomplished by comparing the position and relative intensity of each peak. The overall appearance and location of major peaks in the sample should correspond with the reference spectrum.

8.10.4 Literature Matches: In the event that the laboratory does not possess a known reference material or that a reference material is commercially unavailable, a recognized literature reference may

suffice as supporting data for indications. The source of the spectral data shall be documented in the case file.

8.10.5 Computer aided searches: A search of possible compounds can be conducted using the computer search algorithms in the instrument software. The results of a computer search are to be used only for the purpose of narrowing the number of possible compounds. Computer searches are just survey tools, limited by library, resolution difference, spectra quality, and sample preparation. The forensic scientist evaluates and interprets the comparison of two spectra.

8.10.6 ATR or Diffuse Reflectance: Spectra obtained using an accessory, such as ATR, shall be compared to spectra also obtained using that accessory. For unknowns, a correction factor may be used to aid in searching a transmission library for spectral comparison. The uncorrected spectra should be compared to that of an uncorrected ATR spectrum of a known reference material, if available.

8.10.7 A positive identification, or indication, recorded refers to the drug reference material used in the comparison.

8.10.8 Mixed FTIR spectra can be used for indications if sufficient spectral details are strong and clearly indicate the drug(s) present. A third test should be used to support identification.

8.11 Report Writing: N/A

8.12 References:

8.12.1 Instrument Software

8.12.2 Laboratory QA Manual

8.12.3 Clarke's Isolation and Identification of Drugs, 2nd Ed.; Clarke, E. G. C., The Pharmaceutical Press, 1986.

8.12.4 Instrumental Data for Drug Analysis. 2nd Ed, Mills III, Terry, and Roberson, J. Conrad. New York, New York: Elsevier Science Publishing Company, 1987.

8.12.5 United States Department of Justice Drug Enforcement Administration, Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations, 5th Edition, Supplemental Document SD-2, 02/09/2006.

9. Gas Chromatography/Mass Spectrometry:

9.1. Scope: Gas Chromatography/Mass Spectrometry (GC/MS) is a specific method of identification for most controlled substances (SWGDRUG Category A). It can be used for qualitative and quantitative analyses. The instrument can be used to perform individual analysis or in conjunction with an autosampler for automated batch analysis. This Test Method is intended to give guidance for proper use and interpretation of GC/MS data.

9.2. Precautions/Limitations:

9.2.1. The GC/MS is capable of generating electron ionization spectra in the range of 0-700 amu. The sampling range used for most drug analysis is generally 40-400 amu.

9.2.2. GC/MS has the capability of separating the components of a mixture and providing spectral data for each component. However, it cannot directly distinguish between optical isomers, or salt forms.

9.2.3. Low molecular weight compounds produce few ions and are not easily analyzed using this method. Additional data may be needed to support identification.

9.2.4. Compounds must be volatile and thermally stable for GC/MS analysis. Some common substances degrade upon introduction to the injection port and give spectral information of related substances rather than the compound originally injected.

9.2.5. It is not always possible to identify the molecular ion in a spectrum. There are some classes of compounds that do not give a molecular ion.

9.2.6. It may be necessary to convert some drugs to their free acid or free base form to achieve good chromatographic results.

9.2.7. Periodic maintenance and inspection of the GC/MS will ensure good analytical results. Simple issues, such as a dirty injection port liner can have a significant effect on sample analysis.

9.3. Related Information:

9.3.1. Appendix 1 – Forms and Worksheets
9.3.2. Appendix 2 – Abbreviations
9.3.3. Appendix 3 – Definitions
9.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
9.3.5. Appendix 5 – Instrument Maintenance
9.3.6. Other Test Methods

9.4. Instruments:

9.4.1. Agilent quadrupole GC/MS or equivalent
9.4.2. Autosampler

9.5. Reagents/Materials:

9.5.1. Capillary GC column: usually a flexible fused silica column 0.25 µm id X 30m.
9.5.1.1. HP-5MS or equivalent
9.5.1.2. HP-1MS, DB -1MS,or equivalent
9.5.1.3. Alternate columns may be used if validated and as needs dictate.
9.5.2. Carrier Gas Ultra High Purity grade compressed helium (99.999% purity)
9.5.3. ACS Certified Solvents –i.e.: MeOH, $CHCl_3$
9.5.4. Consumables for the instrument
9.5.5. Autosampler syringes/manual syringes
9.5.6. Autosampler vials and caps
9.5.7. PFTBA (Perfluorotributylamine)
9.5.8. Restek Standard Test Mix (Reference Material Test Mix) or other approved mixture of Reference Materials

9.6. Hazards/Safety:

9.6.1. Solvent/chemical exposure
9.6.1.1. Wash solvents
9.6.1.2. PFTBA
9.6.2. Burns – hot injection port, oven, transfer line, etc.
9.6.3. High pressure carrier gas
9.6.4. Gas cylinder safety concerns
9.6.5. Electrical/Shock hazards

9.7. Reference Materials/Controls/Calibration Checks:

9.7.1. Each instrument shall be autotuned on a weekly basis at a minimum using Perfluorotributylamine (PFTBA) as an autotune reference standard.

9.7.2. A full autotune or equivalent shall be performed. Full autotunes shall be an “A-tune” or an “S-tune”.

9.7.3. A satisfactory autotune shall be when:

9.7.3.1. Mass assignments of m/z 69, 219, and 502 shall be +/- 0.2 amu.

9.7.3.2. Peaks are symmetrical, smooth and the widths shall be between 0.45 and 0.65 amu.

9.7.3.3. There should be less than 200 peaks in the autotune and the mass abundance of the 69 peak should be between 200,000 and 400,000.

9.7.3.4. The tune shall be evaluated for leaks. Peaks at 18, 28 and 32 amu indicate that there may be leaks in the system. Manufacturers' recommendation is that the nitrogen (28) peak be 10% or less.

9.7.3.5. The EM volts should be monitored for increases and decreases.

9.7.3.6. Ion ratios should be in the below listed ranges:

m/z 69	should be the base peak
70/69	≥0.5 but ≤1.6
219/69	≥40% but ≤85%
220/219	≥3.2 but ≤5.4
502/69	≥2.0% but ≤5%
503/502	≥7.9 but ≤12.3

9.7.4. To monitor the instrument performance, a weekly check using a test mixture consisting of three or more drug reference materials (Reference Material Test Mix) shall be run using a temperature program. The chromatogram shall be examined for peak shape, height, and retention time reproducibility as compared to another performance check of the same mixture that was previously run. Additionally the mass spectra of the peaks shall be examined and evaluated.

9.7.5. When a new batch of a test mixture is used, it first must be run twice to demonstrate repeatability for that test mixture. It then can be used weekly to monitor the instrument performance.

9.7.5.1. The test mixture and blank before the test mix must be evaluated for each individual instrument. The blank does not need to be printed, but shall be saved electronically.

9.7.6. Performance checks shall be considered satisfactory upon the distinct separation of the components in the Reference Material test mix and the concentration is consistent with the last acceptable performance check of that test mixture.

9.7.7. If a performance check or calibration is unsatisfactory, the instrument shall be clearly marked and placed out-of-service until acceptable instrument performance has been restored.

9.7.8. When the instrument has undergone repair or has been out of the control of the laboratory for any reason (i.e. shipped out for repair), performance

checks shall be run to ensure proper operation before analysis resumes on that instrument.

9.7.9. Additional maintenance may be performed as needed. Manufacturer's recommendations and/or laboratory practices may specify the frequency of maintenance procedures. See the Instrument Manuals and Appendix 5 for more information.

9.8. Procedures/Instructions:

9.8.1. Instrument Set-up: The instrument conditions (column conditions, carrier gas flow, split or split-less injection mode) should be set to maximize the chromatographic and mass spectral data to be derived from the sample run.

9.8.1.1. The temperature range used is dependent on the length of the column, column flow, and the temperature limits of the column.

9.8.1.2. Capillary GC/MS analysis can be used in either split or split-less injection modes.

9.8.1.3. Either constant pressure or constant column flow of the carrier gas may be used.

9.8.1.4. Isothermal temperature conditions will suffice for most single component drug samples.

9.8.1.5. When dealing with unknowns with no supporting analytical data, a general screening temperature program shall be used.

9.8.2. Sample Preparation:

9.8.2.1. Solid or liquid samples should be dissolved or diluted in methanol or chloroform, as appropriate.

9.8.2.2. Liquid samples may be run as headspace samples.

9.8.2.3. Samples may be placed in autosampler vials, capped and run on the autosampler.

9.8.2.4. Autosampler vials shall be labeled with the appropriate identifiers.

9.8.3. Procedure:

9.8.3.1. Blanks (negative controls) shall be run before and/or between each sample. Reference materials are to be treated as samples and are required to have their own blanks. Any time a sample needs to be re-run, it is not necessary to re-run it's blank if the same sample is re-run immediately. No other injections can be made between back-to-back sample injections.

9.8.3.2. A solvent blank consisting of the solvent used to dissolve the sample shall be run using the same temperature parameters as the sample, including temperature range, ramp rate and hold times. (See 9.9.5.3)

9.8.3.3. Dissolve the unknown sample and/or reference material in a suitable solvent (usually methanol or chloroform) and inject 1-2 µl into the gas chromatograph/mass spectrometer.

9.8.3.4. Test Mixes are considered to be "performance checks" and do not require a blank.

9.8.4. If GC/MS is used as a confirmatory test, then GC retention time cannot be used routinely as a second test to support confirmation.

Supervisory approval is required for use of retention time as a second test, unless it is being used to distinguish between isomers. (See 9.9.1.3) This may be used in situations where other analytical options will not provide useful information, are not possible or available. (Also see Test Method 15 – Synthetic Drugs)

9.9. Records:

9.9.1. Methods: All general GC/MS Methods shall be archived and maintained by the laboratory.

9.9.1.1. If a GC/MS method is modified and saved, a new printout shall be generated listing the parameters, dated, and maintained in a Methods binder.

9.9.1.2. Old methods that are not being used shall be maintained either in a Methods binder or in an archive binder.

9.9.1.3. Any modification to an existing acquisition method shall be noted on the Examination Worksheet.

9.9.2. Maintenance: Each GC/MS instrument shall have a maintenance log.

9.9.3. The status of any instrument that is out of service shall be recorded in the maintenance log as "out of service". The return to service shall be

recorded after satisfactory performance and/or calibration checks have been performed.

9.9.4. Autotunes and Performance Checks: All calibration and performance check data shall be recorded in the instrument maintenance log for each respective instrument. (See Appendix 1)

9.9.4.1. Performance checks and calibration evaluation results shall be indicated in the maintenance log and initialed.

9.9.5. GC/MS Data: GC/MS data, including sample data, solvent blanks, reference blanks, and reference material spectra used for comparison shall be printed, appropriately labeled, and included in the case notes. It is permissible to use the library search information with the comparison of the unknown and known reference spectra.

Additional sample and blank runs that are not used in comparison shall be retained in hardcopy form in the case file and/or stored electronically. If stored electronically, the data shall be retained on the instrument hard drive and/or external hard drive. If data cannot be stored, a Unit Supervisor shall be contacted to discuss alternative methods for storage. Data files shall not be over-written.

If a blank is re-scaled to have a similar abundance as the sample, both the original and re-scaled blanks shall be included.

Existence of multiple sample runs, as well as the reasons for the multiple runs, shall be noted in the case notes.

9.9.5.1. The GC/MS spectra shall be labeled with the name of the instrument (or other unique identifier), the program (method) name and/or general parameters. Multiple runs of blanks and samples shall be identified as such, on the Total Ion Chromatogram, at a minimum. If a sample is run multiple times, the analyst shall document which blank(s) are associated with which run(s) and the data from each run shall be labeled with a Run number. The analyst shall also document which temperature program (or temperature range) and instrument each run was on, if they differ from run to run (e.g. Run 1, Run 2, etc.).

9.9.5.2. Each page of the GC/MS data shall be labeled with the lab file and item numbers, and the hand-written initials of the examiner.

9.9.5.3. The GC conditions (column type, length, and temperature program) shall be indicated on the Examination Worksheet, unless it is specified on the printed GC/MS data.

9.9.5.4. The solvent used to dissolve the sample shall be documented in the preparation section and the GC/MS section on the Examination Worksheet, the GC/MS blank, and the Total Ion Chromatogram.

9.9.5.5. The Examination Worksheet, at a minimum, shall indicate how the samples were prepared.

9.9.5.6. All significant peaks in the Total Ion Chromatogram (all peaks greater than 10% of the most abundant peak in the chromatogram) should be printed and labeled with an evaluation such as identified or unidentified or other similar verbiage.

9.9.5.7. Peaks less than 10% should be evaluated, and printed if deemed relevant.

9.9.5.8. A positive identification or indication recorded refers to the drug reference material used in the comparison.

9.9.5.9. Results of GC/MS data comparison that are being reported (either confirmed or indicated) shall be recorded on the Examination Worksheet.

9.9.5.10. The reason for the additional sample runs shall be noted in the case file.

9.9.5.11. The data from multiple runs shall be included when the results have changed (not because of concentration, chromatography, etc.). Both runs also need to be included if the sample is run on different temperature programs and no peaks are identified. These runs are not considered rejected, unless marked as such.

9.9.5.12. If a sample is re-run due to the presence of a peak in the blank following the sample run, that blank shall be included in the case file. If applicable, the different case number on the following blank does not need to be omitted.

9.9.5.13. The source and lot number of the reference materials used for identification shall be documented in the case file.

9.9.5.14. Each reference material spectra in the user generated spectral library shall be labeled with the source and lot number of the reference material.

9.9.5.15. Retention time data and conclusions shall be recorded in the analysis notes. It is also recommended that the acceptable range (+/- 0.050 minute) be recorded. The retention time of the

reference material and the sample shall be documented on the Examination Worksheet.

9.10. Interpretations of Results:

9.10.1. The sample spectra shall be compared against reference material spectra and/or searched against a user-generated reference material library.

9.10.2. Spectra shall be evaluated by comparing the molecular ion and base peak, and significant ions in the spectrum to that of the known reference material.

9.10.3. Spectra that have significant ions, above the molecular weight of the compound being analyzed are not acceptable, except if:

9.10.3.1. The ions are normally expected isotopic ions or

9.10.3.2. The ions are known contaminant ions:
147, 177, 191, 193, 197, 207, 221, 253, 281, 295, 315, 325, 327, 331, 341,346, 355, 369, 377, 383, 387, 389, 399, 401, 405, 415, 429, 439, 447, 451, 461, 470, 475, 479, 489, 491, 497, 499.
The ions shall be identified and labeled as “column ions” or similar verbiage.

9.10.4. Spectral subtraction is also acceptable if the following data is included: the original spectrum, the spectrum being subtracted, and the resulting spectrum. These shall be clearly labeled on the data.

9.10.5. Positive identification requires comparison of the sample spectrum to a mass spectrum of a verified reference material that has been run on the same instrument.

9.10.6. Samples that degrade in the instrument shall have other supporting data or information to support identification.

9.10.7. Some compounds degrade or lose water during GC/MS analysis and may not have a molecular ion present. Degradation peaks may appear early in the gas chromatogram.

9.10.8. Retention time: The retention time of the unknown peak shall be within (+/-) 0.050 minute of the retention time of the drug reference material.

9.10.8.1. Peak heights of the reference material and unknown sample should be approximately of equal abundance when using a computerized data system or the same integrator settings.

9.10.8.2. The unknown sample and the reference material used for the retention time must be run within 24 hours. It is preferable for these runs to be as close as practical.

9.10.8.3. The manufacturer and lot number of the reference material used for retention time comparison shall be documented in the analytical notes or on the data.

9.11. Report Writing: Substances that degrade and have additional testing procedures to support identifications, or indications, may require alternative wording.

9.12. References:

9.12.1. GC/MS Operator Manuals – Agilent Technologies

9.12.2. Laboratory QA Manual

9.12.3. Clarke's Isolation and Identification of Drugs. 2nd Ed. Clarke, E. G. C., King of Prussia, Pennsylvania, The Pharmaceutical Press, 1986.

9.12.4. Clarke's Analysis of Drug and Poisons, 3rd Edition; Clarke, E. G. C., London, Pharmaceutical Press, 2004.

9.12.5. Instrumental Data for Drug Analysis. 2nd Ed., Mills III, Terry, and Roberson, J. Conrad. New York, New York: Elsevier Science Publishing Company, 1987.

9.12.6. United States Department of Justice Drug Enforcement Administration, Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations, 2nd Edition, Supplemental Document SD-2, 02/09/2006.

10. Gas Chromatography-Infrared Spectroscopy

10.1 Scope: Gas Chromatography-Infrared Spectroscopy (GC-IR) is a combination technique that utilizes the separation capability of the gas chromatograph and the specificity of Fourier Transform Infrared Spectroscopy for the purpose of identifying controlled substances. The DiscovIR GC-IR separates components of complex mixtures in the gas chromatograph (GC), then deposits and freezes each component onto a ZnSe (Zinc Selenide) disk. The instrument then uses an infrared microscope to focus radiant energy through the frozen material to produce solid phase transmission spectral data capable of providing specific chemical and structural information of each substance. It is particularly useful for determining and differentiating between structural isomers of substances. This Test Method is intended to give guidance for proper use and interpretation of GC-IR data.

10.2 Precautions/Limitations:

10.2.1 GC-IR requires liquid nitrogen to cool the detectors. Safety precautions must be taken while handling liquid nitrogen.

10.2.2 A warming detector may lead to poor spectral quality or loss of data.

10.2.3 Disk temperatures may need to be set at lower points for substances such as methamphetamine to improve retention of material on the disk and overall chromatographic quality.

10.2.4 Disk speeds can affect the quality of peak chromatography. Fast speeds cause peak broadening. Slower speeds can cause peaks to co-elute.

10.2.5 The position of the GC column tip may need to be adjusted routinely. Misalignment of the tip will affect chromatography and retention time.

10.2.6 The disk in the GC-IR can accommodate approximately 72 hours of run time before it will need to be cleaned.

10.2.7 As with the GC/MS, the injection port liner, o-ring, etc. will need periodic maintenance to ensure good analytical results.

10.2.8 Compounds must be volatile and thermally stable for GC-IR analysis. The same substances that degrade upon introduction to the injection port on a GC/MS will do the same on the GC-IR. (See also 9.2.4)

10.2.9 Samples concentrations of approximately 1.5-2 mg/ml are necessary to give quality spectral data. This technique may not be appropriate for most residues.

10.2.10 The spectra generated from the DiscovIR are transmittance infrared spectra and can be directly compared with published transmittance data. Care should be taken to note that some spectra produced by GC-IR may not be comparable to published FTIR data because the sample is no longer in a salt form (e.g. Cocaine HCl and Cocaine base will both give the same spectrum (Cocaine base)).

10.2.11 The GC-IR can distinguish between most positional and structural isomers.

10.2.12 This technique cannot distinguish between salt forms of substances, or optical isomers.

10.3 Related Information:

10.3.1 Appendix 1 – Forms and Worksheets
10.3.2 Appendix 2- Abbreviations
10.3.3 Appendix 3- Definitions
10.3.4 Appendix 4- Drug Unit Reagent Preparation Manual
10.3.5 Appendix 5 – Instrument Maintenance
10.3.6 Other Test Methods

10.4 Instruments:

10.4.1 Agilent 7890 Gas Chromatograph
10.4.2 Spectra Analysis DiscovIR direct deposition and detection system, capable of recording spectral data in the mid-IR range of approximately 4000-650cm^{-1}.
10.4.3 Autosampler

10.5 Reagents/Materials:

10.5.1 Capillary GC Column; usually a flexible fused silica column 0.25µm id x15m.
- **10.5.1.1** DB-35MS or equivalent
- **10.5.1.2** HP-1MS, DB-1MS or equivalent
- **10.5.1.3** HP-5MS, DB-5MS or equivalent
- **10.5.1.4** Alternate columns may be used if validated and as needs dictate.

10.5.2 Carrier Gas: Ultra High Purity compressed Helium (99.999% purity)
10.5.3 Liquid Nitrogen
10.5.4 ACS grade solvents – i.e. MeOH, $CHCl_3$, Acetone
10.5.5 Consumables for the instrument
10.5.6 Autosampler syringes
10.5.7 Autosampler vials and caps
10.5.8 Polystyrene (internal to the IR)

10.5.9 Restek Standard Test Mix (Reference Material Test Mix) or other approved mixture of Reference Materials.

10.6 Hazards/Safety:

10.6.1 Solvent/chemical exposure
- **10.6.1.1** Liquid nitrogen
- **10.6.1.2** Wash solvents

10.6.2 High pressure carrier gas
10.6.3 Gas Cylinder safety concerns
10.6.4 Burns- hot injector port, oven and exposed transfer line, etc.
10.6.5 Electrical/Shock hazards

10.7 Reference Materials/Controls/Calibration Checks:

10.7.1 Performance checks shall be performed weekly:
- **10.7.1.1** A voltage check,
- **10.7.1.2** A noise check,
- **10.7.1.3** A polystyrene Reference Material check,
- **10.7.1.4** A Test Mix/Reference Material Mix check

10.7.2 Satisfactory checks shall be when:

10.7.2.1 Voltage checks are between 3 and 7 volts.

10.7.2.2 Noise checks are between 0.4 and 1.0 mABS.

10.7.2.3 Polystyrene checks are +/- 2.5 cm^{-1} of the following bands:

Band 1	3060.2 cm^{-1}
Band 2	1601.5 cm^{-1}
Band 3	1583.2 cm^{-1}
Band 4	1028.5 cm^{-1}
Band 5	906.7 cm^{-1}

10.7.3 Test Mix check shall be examined for chromatographic peak shape, height and retention time reproducibility as compared to another performance check of the same mixture that was previously run. (See also 9.7.5 and 9.7.6)

10.7.3.1 Retention times must be within (+/-) 0.15 min for satisfactory reproducibility of the Test Mix.

10.7.3.2 Additionally the IR spectra of the each component of the Test Mix shall be examined and evaluated.

10.7.4 If any of the performance checks are found to be outside their designated ranges or otherwise found to be unsatisfactory, the

instrument shall be clearly marked and placed out of service until satisfactory performance of the instrument has been restored.

10.8 Procedures/Instructions:

10.8.1 Check Performance/Operating Procedure:

10.8.1.1 Cool Instrument by adding liquid nitrogen to the detector and the disk.

10.8.1.2 Settings should be:

10.8.1.2.1 Vacuum pressure should be below 9 X 10^{-3} torr.

10.8.1.2.2 Transfer Line*, Restrictor and Oven Temperatures should be set at the same temperature.

These values should be (+/-) 10 degrees of the start point.

*This should be set at low temperatures during periods of down time to extend the life of the transfer line.

10.8.1.2.3 Disk Temp: below -30°C

10.8.1.2.4 Resolution: 4cm^{-1} (Manually set/permanent setting)

10.8.1.2.5 Split Ratio: 10:1 (recommended)

10.8.2 Voltage and Noise Check Procedures: (See GC-IR Maintenance Manual). Record these values on the Calibration Verification log.

10.8.3 Polystyrene Check: (See GC-IR Maintenance Manual). Print the spectrum and record the check on the Calibration Verification log.

10.8.4 Test Mix Performance Check: Print the background, the Test Mix blank, and chromatogram of the Test Mix. These shall be kept in the instrument Calibration Verification log.

10.8.5 Sample Criteria for GC-IR analysis:

10.8.5.1 Any substance that requires isomer determination because it is specifically named as a specific isomeric form in the Indiana Criminal Code,

10.8.5.2 The substance is not controlled by structure, AND

10.8.5.3 The reference materials for all existing positional isomers are not available for comparison, OR

10.8.5.4 Analyses of the various positional isomers do not produce a conclusive identification.

10.8.6 Sample Preparation:

10.8.6.1 Samples should be dissolved in a suitable solvent, such as $CHCl_3$, MeOH and/or Acetone.

10.8.6.2 Samples may be placed in autosampler vials, capped and run on the autosampler.

10.8.6.3 Autosampler vials shall be labeled with the appropriate identifiers.

10.8.7 General Operating Procedure:

10.8.7.1 A solvent blank consisting of the solvent used to dissolve the sample shall be run within the same temperature range of the sample.

10.8.7.2 Solvent blanks shall be run before each sample in the same location where the sample is to be deposited on the ZnSe disk.

10.8.7.3 Approximately 1-2 µL of sample is to be injected using an autosampler. Recommended sample concentrations should be approximately 1.5-2.0 mg/mL.

10.9 Records:

10.9.1 Methods: All methods shall be archived and maintained in the Laboratory.

10.9.1.1 If a GC-IR method has been modified and saved, a new printout shall be generated listing the parameters, dated, and maintained in a Methods binder.

10.9.1.2 Old methods that are not being used shall be maintained either in a Methods binder or in an Archive binder.

10.9.1.3 Any modification to an existing acquisition method shall be noted on the Examination Worksheet.

10.9.2 Maintenance: Each instrument shall have a maintenance log.

10.9.3 The status of any instrument that is out of service shall be recorded in the maintenance log as “out of service”. The return to service shall be recorded after satisfactory performance and/or calibration checks have been performed.

10.9.4 Performance Checks: All calibration and performance check data shall be recorded on the instrument Calibration Verification log.

10.9.4.1 Performance checks and calibration verification evaluation results shall be indicated on the Calibration Verification log and initialed.

10.9.4.2 The evaluation and acceptance of the FTIR spectral data associated with the Test Mix shall be documented on the Calibration Verification log.

10.9.5 GC-IR Data: GC-IR data, including sample data, solvent blanks, reference blanks and reference material spectra used for comparison shall be printed, appropriately labeled and included in case notes. It is permissible to use the library search information with the comparison of the unknown and known reference spectra.

Additional sample and blanks runs that are not used in comparison shall be retained in hardcopy form in the notes and/or stored electronically. If stored electronically, the data shall be retained on the instrument hard drive and/or external hard drive. If data cannot be stored, a Unit Supervisor shall be contacted to discuss alternative methods for storage. Data files are not to be over-written.
Existence of multiple runs and the reasons shall be included in the case file.

10.9.6 GC-IR data shall be labeled with the name of the instrument (or other unique identifier), the program (method) name and/or general parameters. Multiple runs of blanks and samples shall be identified as such, on the Absorbance Chromatogram, at a minimum (e.g. Run 1, Run 2, etc.). The data from each run shall be labeled with a Run number.

10.9.7 GC conditions such as column type, length and temperature program shall be indicated on the Examination Worksheet, unless this information is specified on the printed GC-IR data.

10.9.8 The solvent used for the blank and sample shall be documented both on the Examination Worksheet and the spectral data.

10.9.9 Each page of the GC-IR data shall be labeled with the lab file and item numbers, and the hand-written initials of the examiner.

10.9.10 All significant peaks in the Absorbance Chromatogram (all peaks greater than 10% of the most abundant peak in the chromatogram) should be printed and either marked as identified or unidentified. Any peak below 10% of the most abundant peak in the chromatogram should be evaluated and labeled with an evaluation such as identified or unidentified or other similar verbiage.

10.9.11 A positive identification or indication recorded refers to the drug reference material used in the comparison.

10.9.12 Results of GC-IR data comparisons shall be recorded on the Examination Worksheet. The reasons for additional sample and blanks runs shall be noted in the case file.

10.9.13 The source and lot number of the reference material(s) used for identification shall be documented in the case file.

10.9.14 Each reference material in the user generated library shall be labeled with the name of the material, the source and lot number of the reference material.

10.9.15 All reference material spectra, and/or user generated library spectra, shall be maintained electronically on the instrument that generated it, in addition to being stored and/or backed up on an external hard drive.

10.10 Interpretation of Results:

10.10.1 Spectra must be well resolved and of a sufficient intensity to permit identification.

10.10.2 Identifications shall be made by direct comparison to a known reference material of the substance being analyzed, and/or and entry from a user-generated library, generated on the same instrument.

10.10.3 Spectral comparison shall be accomplished by evaluating the overall appearance of the sample spectrum and position of major peaks as it compares with a known reference material.

10.10.4 Literature Matches: In the event that the laboratory does not possess a known reference material or that a reference material is commercially unavailable, a recognized literature reference may suffice as supporting data for indications. The name of the literature source shall be included in the case file.

10.10.5 In the absence of published literature, spectral data from another accredited laboratory may be used as supporting data for indications of identity.

10.10.6 Verification of reference material spectra. (See 31.8.6) If published spectra are not available, spectral data from two other accredited laboratories may be used as verification of reference material spectra, or another method of verification of the standard may be used in combination with spectral data from one independent accredited lab.

10.11 Report Writing: N/A

10.12 References:

10.12.1 DiscovIR Operating Manual, Spectra Analysis, Rev B., June 2011

10.12.2 Gas-Chromatography-Infrared Spectroscopy Validation, (Indiana State Police), Roskowski, Newton and Yovanovich, 2013.

10.12.3 GC-IR Operators Instructions, (Indiana State Police) Yovanovich, 2013

11. Polarimetry:

11.1. Scope: Polarimetry is a SWGDRUG Category C test and one of two methods used in the Indiana State Police Laboratory to determine the optical isomer of optically active compounds. Optical activity of submitted samples is measured by passing plane polarized light through a solution containing the sample. There are some substances that have only one optical isomer or racemic mixture that is controlled, whereas the remaining isomer is not. The specific isomeric form must be determined, if possible, in these cases for charges to be filed against the accused. This Test Method is intended to provide instruction for the proper use and interpretation of Polarimetry data.

11.2. Precautions/Limitations: The magnitude of rotation is dependent on several factors:

11.2.1. The temperature of the solution. Experimental values will vary due to inability to maintain temperatures specified in literature.

11.2.2. The concentration of the solution will affect the magnitude of the rotation. Sample and reference materials should be compared at similar concentrations.

11.2.3. Wavelength of the light used in the analysis.

11.2.4. The path length (of the cell) the light travels through the sample.

11.2.5. It is essential to have optically pure reference materials and optically purified unknown samples. Mixtures of optically active substances will lead to incorrect results. It may be necessary to extract the sample.

11.2.6. Nature of the solvent is important. This information is specified in literature.

11.2.7. The properties of the compound being subjected to analysis. The correct salt or free base form is necessary for polarimetry analysis and comparison with literature values. Mixtures with optically inactive substances do not interfere with polarimetry analysis.

11.3. Related Information:

11.3.1. Appendix 1 – Forms and Worksheets
11.3.2. Appendix 2 – Abbreviations
11.3.3. Appendix 3 – Definitions
11.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
11.3.5. Other Test Methods
11.3.5.1. General Drug Analysis
11.3.5.2. Reference Materials

11.4. Instruments: Polarimeter

11.5. Reagents/Materials:

11.5.1. Dextropropoxyphene Reference Material
11.5.2. Dextromethorphan Reference Material
11.5.3. Other reference materials, as procedures are validated.
11.5.4. Chloroform ($CHCl_3$)
11.5.5. Distilled water
11.5.6. 10ml volumetric flask
11.5.7. Pipettes
11.5.8. Polarimetry cell

11.6. Hazards/Safety:

11.6.1. Chemical exposure to $CHCl_3$, Dextropropoxyphene, and other drugs.

11.7. Reference Materials/Controls/Calibration Checks:

11.7.1. The dextropropoxyphene (base) performance check solution shall be made according to the following specifications: 0.6 gram Dextropropoxyphene base in 100 ml of chloroform.

11.7.2. The performance of the polarimeter is verified on the day of analysis using dextropropoxyphene (base). (See 11.9.)

11.7.3. A 1 dm cell and sodium lamp shall be used. The calculated rotation of dextropropoxyphene would be + 0.404.

11.7.4. Observed rotation shall be $\pm$ 0.1 from the calculated rotation.

11.7.5. If the observed rotation of the performance check solution is outside the acceptable limits, it shall be discarded. The solution shall be re-made and verified.

11.7.6. A solvent blank shall be run before and after each reference material. This provides verification that the polarimetry cell and solvent are not contaminated.

11.7.6.1. If the solvent blank is not satisfactory, the cell shall be cleaned and the blank re-run. If the cell cannot be cleaned, it shall be replaced.

11.7.6.2. Maintenance: The polarimeter has no routine maintenance. In the event of a source failure or malfunction, it shall be

replaced. If the instrument repeatedly fails its performance checks, it shall be taken out of service and repaired.

11.8. Procedures/Instructions:

11.8.1. Polarimetry measurements shall be determined for the optically pure drug reference material (dextro, levo, or both) and the purified unknown drug.

11.8.2. A solvent blank shall be run before and after each sample or reference material. This provides verification that the polarimetry cell and solvent are not contaminated.

11.8.3. Run the performance check solution. (See 11.7.1).

11.8.4. Dissolve extracted sample in $CHCl_3$, or suitable solvent.

11.8.5. Place sample solution in the polarimetry cell and obtain sample rotation value.

Absolute rotation and specific operating conditions for optical isomer determination of optically active drugs are available in references such as The Merck Index.

11.9. Records:

11.9.1. Maintenance: Each polarimeter instrument shall have a maintenance log.

11.9.2. The performance checks, including observed rotation of the reference material and solvent blank, shall be documented in the instrument maintenance log. The source and lot number of the reference material used and date shall be noted.

11.9.3. The status of any instrument that is out of service shall be recorded in the maintenance log as “out of service”. The return to service shall be recorded after satisfactory performance and/or calibration checks have been performed.

11.9.4. The observed degree of rotation for the reference material, unknown, and a solvent blank, as well as a conclusion as to dextro, levo or racemic isomer form for the unknown drug shall be recorded in the notes.

11.10. Interpretations of Results:

11.10.1. A determination for optical isomeric form (dextro or levo) will be based on a positive or negative rotation of plane polarized light by the unknown sample.

Rotation in the positive direction (+) identifies the dextrorotatory isomer.

Rotation in the negative direction (-) identifies the levorotatory isomer.

No rotation indicates an optically inactive compound or a racemic mixture.

11.10.2. The solvent blank should show no rotation of plane polarized light.

11.11. Report Writing:

11.11.1. If the observed rotation is determined to be the dextrorotatory isomer, it shall be reported as the dextro or d-, isomer.

11.11.2. If the levorotatory isomer is identified, it shall be reported as the levo or l-, isomer.

11.11.3. If the optical isomer has not been determined, the report shall reflect the drug name without reference to its isomeric form (e.g. Propoxyphene or Methorphan) and “the specific isomer was not determined” statement shall be included in the report. If the difference in isomeric form (dextro- vs levo-) results in a change of control status, the control status shall be omitted from the report.

11.12. References:

11.12.1. Merck Index

11.12.2. Drug Resource Manual

11.12.3. Drug Unit Training Manual

11.12.4. Laboratory QA Manual

12. Mixed Melting Point Determination:

12.1. Scope: Melting Point Determination is a SWGDRUG Category C Test and another method of optical isomer determination used by the Indiana State Police Laboratory. This method is capable of determining the isomeric form of a wide range of compounds. This Test Method is intended to provide instruction for the proper use and interpretation of Mixed Melting Point data.

12.2. Precautions/Limitations:

12.2.1. It is essential to have optically pure drug reference material and purified unknown samples. The presence of other substances may cause an incorrect interpretation of results. Familiarization with literature reference values is essential.

12.2.2. The salt form of both the reference material and the unknown sample shall be the same.

12.2.3. The substances being analyzed must be fully dry, homogenous and in powdered form.

12.2.4. The powder needs to be finely ground. The efficiency and reproducibility of the heat transfer into the sample is dependent on it.

12.2.5. The unknown and the known isomeric form of the reference material shall be mixed, dissolved in an appropriate solvent and re-crystallized.

12.2.6. Temperature increases must be gradual. A fast ramp causes the sample to melt quickly and results can be misinterpreted, inaccurate, or altogether missed.

12.2.7. A mixture of levo and dextro isomers does not always result in a melting point depression, or lowering of the melting point.

12.3. Related Information:

12.3.1. Appendix 1 – Forms and Worksheets
12.3.2. Appendix 2 – Abbreviations
12.3.3. Appendix 3 – Definitions
12.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
12.3.5. Other Test Methods
11.3.5.1 General Drug Identification
11.3.5.2 Reference Materials

12.4. Instruments: Melting Point Apparatus

12.5. Reagents/Materials:

12.5.1. Melting point capillary tubes
12.5.2. Appropriate Reference Materials

12.6. Hazards/Safety:

12.6.1. Burns
12.6.2. Broken glass
12.6.3. Chemical exposure – heated fumes, general drug

12.7. Reference Materials/Controls/Calibration Checks:

12.7.1. If both dextro and levo isomers of the drug reference material are available, then a mixed melting point determination of the reference material (50:50 ratio of isomers) should be conducted to demonstrate that the melting point is actually depressed upon mixing the two isomers. Racemic mixtures of isomers do not always exhibit a depression in melting point. Consult literature for specific drug melting point information.

12.7.2. The melting point apparatus shall be verified before or at the time of each use using appropriate reference materials (optically active reference materials for drug of interest in the examination.) (See 12.9.2)

12.7.3. The observed beginning and ending melting temperature (melting point range) will be within ± 1 degree Celsius of the expected temperature range.

12.7.4. Maintenance: The melting point apparatus has no routine maintenance procedures. Temperatures are monitored at the time of use and are compared against the known melting points of the substances being analyzed. If the instrument fails to perform as expected, it shall be taken out of service, evaluated and repaired or replaced, as necessary.

12.8. Procedures/Instructions:

12.8.1. A 50:50 mixture of optically pure drug reference material (either dextro or levo) and the purified unknown drug should be prepared. The mixture shall be dissolved in a suitable solvent and re-crystallized.

12.8.2. The melting points of the optically pure reference material; purified unknown; and a 50:50 mixture of the reference material and unknown shall each be determined using a melting point apparatus.

12.8.3. Place sample, reference material and the 50:50 reference material: sample mixture into melting point capillary tubes. The sample height should be approximately 2-3mm for optimum results.

12.8.4. Place each capillary tube into a slot in the Melting Point apparatus.

12.8.5. Heat the apparatus at a slow rate.

12.8.6. Observe and record the temperatures at which the substances begin to melt and when the substances are completely melted.

12.9. Records:

12.9.1. All melting points should be recorded as a range between the onset of melting (Onset point) and the point at which the last crystal has melted (the clear or liquefaction point).

12.9.2. Maintenance: Each melting point apparatus shall have a maintenance log. .

12.9.3. The status of any instrument that is out of service shall be recorded in the maintenance log as “out of service”. The return to service shall be recorded after satisfactory performance and/or calibration checks have been performed.

12.9.4. The melting points for the reference material, unknown, and mixture shall be recorded in the notes with a conclusion as to the optical form for the unknown drug (dextro, levo, or racemic).

12.10. Interpretations of Results:

12.10.1. The dextro and levo isomers are expected to melt at the same temperatures. Generally, a 50:50 mixture of these isomers is expected to give a depressed melting point. This is not always true and depends on the substance being analyzed. Consult literature information for specific drug information.

12.10.2. If the melting point of the sample is consistent with the melting point of a single isomer, it can be concluded that it is a relatively pure single isomer.

12.10.3. If the melting point of the sample is not consistent with the melting point of a single isomer, it is possible that either the sample exists as a racemic mixture or that the sample is not pure.

12.10.4. If the temperature range of the 50:50 mixture of the reference material and the unknown sample is consistent with the melting point of one isomeric form, then the unknown sample can be concluded to be the same isomer as the known reference material in the mixture.

(i.e. If the unknown sample is mixed with the d-isomer and the resulting melting point is consistent with the melting point of a single isomeric form, then the isomeric form of the unknown is the dextro, or d – form of the drug.)

12.10.5. If the temperature range of the 50:50 mixture of the reference material and the purified unknown sample is consistent with the depressed melting point of a mixture of dextro and levo isomers or lower than the melting point of one isomeric form, then the unknown sample can be concluded to contain the opposite isomer as the known reference material in the mixture. (See 12.2.1 for precautions.)

(i.e. If the unknown sample is mixed with the d-isomer and the resulting melting point differs from the known melting point of one isomeric form and is consistent with the melting point of the racemic mixture, then two isomeric forms are present. It can then be concluded that the unknown is of the opposite isomer as the known reference material and would be the levo, or l, isomer.)

A careful evaluation of the known, unknown and 50:50 sample/unknown mixtures is essential to avoid a misinterpretation due to impurities and/or racemic mixture.

12.11. Report Writing:

12.11.1. Isomeric forms, if determined, shall be reported as the dextro or levo isomers.

12.11.2. If it has been determined that a racemic mixture is present, the item shall be reported as a racemic mixture, or its common name, whichever is most appropriate to clarify the results.

12.11.3. If the optical isomer has not been determined, the report shall reflect the drug name without reference to its isomeric form (e.g. Propoxyphene or Methorphan) or its control status, and “the specific isomer was not determined” statement shall be included in the report. If the difference in isomeric form (dextro- vs levo-) results in a change of control status, the control status shall be omitted from the report.

12.12. References:

12.12.1. Drug Unit Resource Manual
12.12.2. Merck Index
12.12.3. Laboratory QA Manual
12.12.4. Melting Point Determination Application Note #1, Stanford Research Systems (www.thinkSRS.com)

13. Separation and Extraction Procedures:

13.1. Scope: Most drugs are often complex mixtures of substances and contain large amounts of diluents and/or adulterants. These additives can interfere with analysis and it is frequently necessary to separate them so that the drug(s) of interest can be identified by analytical methods. A variety of separation or purification procedures can be used to purify drugs including, but not limited to: liquid-liquid extractions, preparative thin layer chromatography, Alternate Non-Aqueous Organic Ratio (ANOR) extractions, solvent dry extractions and column chromatography. This Test Method is not an all inclusive list of the acceptable extractions, but rather a guide for such procedures.

13.2. Precautions/Limitations:

13.2.1. Extraction procedures require a sufficient sample size to perform the test. This may result in a significant loss of sample.

13.2.2. Clean glassware must be used. Dirty glassware can be a source of contamination.

13.2.3. Extraction procedures may convert the sample form of the analyte to its free base or acid, or cause sample decomposition.

13.2.4. Some extracted drugs are volatile and will evaporate unless converted to a stable form.

13.3. Related Information:

13.3.1. Appendix 1 – Forms and Worksheets
13.3.2. Appendix 2 – Abbreviations
13.3.3. Appendix 3 – Definitions
13.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
13.3.5. Other Test Methods

13.4. Instruments: Centrifuge and/or vortex, if necessary.

13.5. Reagents/Materials:

13.5.1. Organic Solvents : $CHCl_3$, Petroleum Ether, Methanol, Hexane
13.5.2. Acids : HCl, H_2SO_4
13.5.3. Bases : NaOH, NH_4OH, Sodium Bicarbonate
13.5.4. Filter Paper
13.5.5. Pipettes
13.5.6. Separatory funnels
13.5.7. Beakers
13.5.8. Culture Tubes
13.5.9. Prep Thin Layer Supplies (See TLC method)

13.6. Hazards/Safety:

13.6.1. Inhalation Hazards
13.6.2. Exposure Hazards
13.6.3. Sharps Hazard

13.7. Reference Materials/Controls/Calibration Checks: When validating or verifying an extraction procedure, the procedure shall be verified by using a known reference material or preparation to demonstrate the performance of the extraction.

13.8. Procedures/Instructions:

13.8.1. The selection of a purification or separation procedure shall be based upon the components of the sample.

13.8.2. Recommended extraction procedures are listed in each drug Test Method.

13.8.3. For compounds not individually listed, extraction and solubility information may be found in references such as the Drug Unit Resource Manual, Clarke's Isolation and Identification of Drugs, Clarke's Analysis of Drugs and Poisons, The Merck Index, and the Physician's Desk Reference. The manufacturer may also supply this information.

13.8.4. Samples shall be dissolved in and extracted with the appropriate solvents.

13.8.5. It may be necessary to add HCl fumes to convert volatile samples to a more stable form.

13.8.6. Fume hoods shall be used when evaporating solvent extracts.

13.8.7. Glassware used to collect extracted samples should be covered while in storage to protect from loss or contamination. Parafilm is sufficient for this purpose.

13.9. Records:

13.9.1. A description of the extraction or purification procedure shall be recorded in the case notes in sufficient detail to be understood and replicated by a trained forensic scientist. It is sufficient to label the spectrum as "extracted", if the details of that extraction are included on the Examination Worksheet or vice versa.

13.9.2. If a sample preparation or extraction procedure is detailed in the relevant Test Method, it is permissible to cite that portion of the Test

Method that contains the details of the extraction. Optional steps shall be noted if they were or were not used in the procedure. (e.g. mushroom sample preparation and extraction)

13.9.3. The tests that have been run using an extracted sample shall be identified in the analytical notes and, if applicable, on the printout of the data.

13.10. Interpretations of Results: N/A

13.11. Report Writing: N/A

13.12. References:

13.12.1. Drug Unit Resource Manual(s)

13.12.2. The ANOR (Alternate Non-Aqueous Organic Ratio) Extraction Procedure, Mary A. Rhodes, Criminalist, Birmingham, Alabama, April 1982.

13.12.3. The ANOR (Alternate Non-Aqueous Organic Ratio) Extraction Procedure, Allen R. Adair, B.S., F. Taylor Noggle, Jr., B.S., Martha S. Odom, B.S., Mary A. Rhodes, B.S., Microgram, Vol. XVI, No.1, 1 January 1983.

13.12.4. Extraction Procedures, William S. Bowles, Memo to J. Forbes, March 1981

13.12.5. Clarke's Isolation and Identification of Drugs, 2nd Edition; Clarke, E. G. C. The Pharmaceutical Press, 1986.

13.12.6. Clarke's Analysis of Drugs and Poisons. 3rd Edition; Clarke, E. G. C. The Pharmaceutical Press, 2004

13.12.7. The Merck Index, 8th Edition; Merck and Company, Inc. 1968

13.12.8. Occurrence of Excipient Materials in Illicit Tablet Manufacture, Rhodes, and Thornton (University of California – Berkeley), Microgram, Vol. XII, No. 5, (May 1979).

14. Marijuana Examination:

14.1 Scope: Suspected Marijuana and/or marijuana preparations are examined visually, macroscopically, and microscopically noting morphological characteristics. Additional tests such as color tests, thin layer chromatography, gas chromatography, and GC/MS are available to be used to identify the components of plant material, hashish, hash oil, and residues.

14.2 Precautions/Limitations:

14.2.1 Immature plants may not have enough developed plant features to permit microscopic identification. Additionally they may not be mature enough to produce enough cannabinoids to detect.

14.2.2 Burnt plant material may not have enough identifiable plant features remaining for microscopic identification.

14.2.3 Finely pulverized material, compressed and/or extracted plant material preparations (i.e. Hashish, baked goods, residues, etc.) pose difficulties in identifying botanical characteristics due to the small size of the material, and the matrices involved.

14.2.4 Hash Oil is very concentrated and needs to be diluted for analysis.

14.2.5 Wet plant material shall not be accepted for analysis. It is the submitting officer's responsibility to dry plant material. Mold not only presents a health and fire hazard, it also obscures the plant features. Long term exposure to moisture contributes to severe degradation of the plant material.

14.2.6 Mature stalks of Marijuana are exempt from the Indiana Criminal Code. If a sample includes plant stalks, and the weight of the plant material is needed, it must be stripped from the stalks. It is the responsibility of the submitting officer to strip the plant material from the stalks.

14.3 Related Information:

14.3.1 Appendix 1 – Forms and Worksheets
14.3.2 Appendix 2 – Abbreviations
14.3.3 Appendix 3 – Definitions
14.3.4 Appendix 4 – Drug Unit Reagent Preparation Manual
14.3.5 Other Test Methods
- **14.3.5.1** General Drug Identification
- **14.3.5.2** Weighing Determinations
- **14.3.5.3** Evidence Handling
- **14.3.5.4** Color (Spot) Testing
- **14.3.5.5** Sampling
- **14.3.5.6** Thin Layer Chromatography

14.3.5.7 Gas Chromatography Mass Spectrometry
14.3.5.8 Gas Chromatography-Infrared Spectroscopy

14.4 Instruments:

14.4.1 Stereomicroscope
14.4.2 GC/MS – for items where plant features may not be visible (e. g paraphernalia, hash oil, hashish, etc.)
14.4.3 GC-IR – for items mixed with synthetic or other drugs.

14.5 Reagents/Materials:

14.5.1 Ceramic well plate
14.5.2 Duquenois reagent/$CHCl_3$/Hydrochloric Acid
14.5.3 TLC supplies (See Thin Layer Chromatography Test Method)
14.5.3.1 Toluene TLC System
14.5.3.2 Diethylamine
14.5.3.3 Fast Blue BB Salt
14.5.4 Germination chamber
14.5.5 Growing Medium
14.5.6 Water
14.5.7 Culture tubes

14.6 Hazards/Safety:

14.6.1 Moldy plant material presents both health and fire hazards. The Aspergillus fungus can cause a condition known as Farmer's Lung, which can be fatal. Wet and moldy plant material generates its own heat and can start a fire if left unattended.

14.6.2 Insects and bugs are commonly found in plant material evidence. Improper packaging can lead to an infestation of the evidence storage facilities.

14.6.3 Chemical Exposures/Inhalation Hazards including potential carcinogens.

14.7 Reference Materials/Controls/Calibration Checks:

14.7.1 Blanks: A blank shall be run for the Duquenois-Levine color test in conjunction with examining evidence.

14.7.2 Controls: See Reagent Preparation Manual and Color (Spot) Tests Method (5.7.3 and 5.9.2)

14.7.3 Thin Layer Chromatography (TLC): Appropriate reference materials shall be run with the blanks and samples. See TLC Test Method (7.7)

14.8 Procedures/Instructions:

14.8.1 All suspected Marijuana items, except residues, ashes, seeds, and cigarette butts, shall be weighed.

14.8.2 Items of plant material can be administratively withdrawn if no additional weight thresholds will be met by the analysis of these items. The unexamined evidence shall be weighed. If the plant material can be seen through the outermost packaging, the evidence does not need to be opened and a gross weight including outermost packaging can be taken.

14.8.3 Microscopic Identification: Suspected Marijuana shall be examined using a stereomicroscope with approximately 7x to 30x powers of magnification for the presence of botanical characteristics such as leaf fragments with both cystolithic and fine hairs, veins on leaves, seeds, multi-cellular hairs, stems, stalk, and flowering tops that are consistent with Marijuana.

14.8.3.1 Suspected Hashish samples can be examined by placing a portion of the sample on a microscope slide with several drops of chloroform, or other suitable solvent, and examined microscopically for the presence of botanical characteristics such as cystolithic hairs, simple hairs, etc. The presence of detached hairs should be noted for hashish samples, if present.

14.8.4 Duquenois-Levine Color Test: The Duquenois-Levine Color Test is used in the examination of Marijuana, Hashish, Hash Oil, THC, and Marijuana residues. This test can be performed directly on a portion of the sample or vegetation in a small test tube, or can be performed on a small amount of petroleum ether or methanol extract of the sample or vegetation in a spot plate or evaporating dish. The extraction procedure shall be documented on the Examination Worksheet.

14.8.5 Thin Layer Chromatography: TLC is sufficient to resolve the three major cannabinoids (Tetrahydrocannabinol, Cannabinol, and Cannabidiol). See TLC Test Method.

14.8.5.1 Unknown samples and cannabinoid reference materials are routinely dissolved in Methanol, or Petroleum Ether.

14.8.5.2 Plates should be sprayed with Diethylamine prior to development to improve separation.

14.8.5.3 Toluene shall be used as the development solvent.

14.8.5.4 Fast Blue BB shall be used as the visualizing spray.

14.8.6 Gas Chromatography/Mass Spectrometry: This is appropriate for paraphernalia, Hash Oil, Hashish, or other situations where plant features are not visible, obscured or absent. A general temperature program should be sufficient. (See GC/MS Test Method)

14.8.7 Gas Chromatography-Infrared Spectroscopy: This technique may be used to analyze plant materials for synthetic and other drugs as necessary.

14.8.8 Tetrahydrocannabinolic Acid (THCA) converts to THC in the injection port. In order to confirm the presence of THC in a sample, TLC must be performed.

14.8.9 If a confirmatory technique (SWGDRUG Category A) is not used, then at least three different methods must be used for identification.

14.8.10 Samples that are negative for cannabinoids should be screened for the presence of other controlled substances by either running a TLC plate in one of the general screening systems (See TLC Test Method) or by GC/MS.

14.9 Records:

14.9.1 All weights used to meet or exceed weight limits of a particular criminal charge shall be recorded as net weight.

14.9.2 Observations of microscopic botanical characteristics shall be recorded on the analysis sheet. (See 14.8.2 and 14.8.2.1)

14.9.3 Duquenois-Levine: Conclusions as to the solvent blank and the color of the reaction(s) of the unknown sample shall be recorded in the notes. Weak or intense reactions should be particularly noted. (See 14.10.3 and 14.10.4)

14.9.4 Thin Layer Chromatography: Conclusions as to the solvent blank and spots in the unknown sample shall be recorded in the analysis notes. Weak or intense reactions should be particularly noted.

14.9.5 If Gas Chromatography/Mass Spectrometry is performed: See GC/MS Test Method.

14.10 Interpretations of Results:

14.10.1 The combination of three analytical techniques must demonstrate the identity of the specific drug (i.e. Marijuana) and must preclude a false positive identification.

14.10.2 A microscopic examination is required to identify plant material as Marijuana.

14.10.3 Hashish is a concentrated resinous material that generally produces strong reactions to the Duquenois-Levine and TLC tests. Microscopically, Hashish may resemble compressed material and exhibit detached botanical features.

14.10.4 Hash Oil also generally produces strong reactions to the Duquenois-Levine and TLC tests, but exists as a thick, viscous liquid and has no macroscopic or microscopic features.

14.10.5 Microscopic examination: Cystolithic hairs and fine hairs should be observed on opposite sides of the same leaf, or leaf fragment. The observations of additional features are supportive.

14.10.6 Duquenois-Levine: A positive result for cannabinoids will be based upon an initial expected color of blue, violet, pink or purple which then extracts into the chloroform layer.

14.10.7 Thin Layer Chromatography: Positive indication of the unknown sample will be based on color and location of spots on the plate relative to the cannabinoid reference material(s) and indicate the cannabinoids(s) present. If there is no indication of Tetrahydrocannbinol on TLC, the sample cannot be reported as Marijuana. Additional testing will be required to confirm the presence of other cannabinoids.

14.10.8 Gas Chromatography/Mass Spectrometry: (See GC/MS Test Method).

14.10.9 Gas Chromatography-Infrared Spectroscopy: (See GC-IR Test Method).

14.11 Report Writing:

14.11.1 If reported in the Certificate of Analysis, weights of marijuana cigarettes and marijuana cigarette butts shall be reported as gross weight.

14.11.2 Marijuana items with a total weight greater than ten (10) pounds shall be recorded in both the notes and reported as grams and pounds.

14.11.3 Hashish and Hash Oil may be reported as such when sufficient observations and analysis support the conclusion of those forms of Marijuana.

14.11.4 Marijuana and Tetrahydrocannabinol (THC) shall not have a control status listed on the Certificate of Analysis.

14.11.5 The wording for reporting Marijuana and Tetrahydrocannabinol (THC) shall follow the wording in the 2019 Marijuana Reporting Directive 19/Lab Div/016 or similar verbiage. However, there shall be no reference to the accreditation status of the outsourcing laboratory.

14.11.6 Cannabinol, Cannabidiol and Tetrahydrocannabinolic Acid shall be reported as federally controlled substances.

14.11.7 In some cases items of plant material will be administratively withdrawn because no additional weight thresholds have been met..

14.11.7.1 If the evidence is not opened, the report shall use the following or similar verbiage:

Item 001 was not analyzed or opened.
The gross weight of item 001, including outermost packaging, was X gram(s).

14.11.7.2 If the evidence is opened and weighed, the report shall use the following or similar verbiage:

Item 001 was not analyzed.
The net (or gross) weight of item 001 was X gram(s).

14.12 References:

14.12.1 Drug Unit Resource Manual – Marijuana

14.12.2 The Botany and Ecology of Cannabis, Robert Connell Clark

14.12.3 The Botany and Chemistry of Cannabis, Joyce & Curry, Chapters 1,2,6 pages 93-99, 111-115, 120-121

14.12.4 Basic Training Program for Forensic Drug Chemists, Canaff, US Department of Justice Bureau of Narcotics and Dangerous Drugs, May 1972.

14.12.5 Controlled Substance Act, pertaining to Marijuana and Hashish

14.12.6 Indiana Criminal Code, IC 35-48-1-19: Definition of Marijuana

14.12.7 Marijuana Thin Layer Chromatography Systems, Memo to J. Forbes, Huttsell, F. (ISP), February, 1991

14.12.8 Marijuana Seed Germination Instructions (SharePoint)

14.12.9 Visual Characteristics of Cannabis Sativa (Marijuana) Seeds; Fussel, Thornton, and Whitehurst, Journal of Forensic Identification 59(5), 2009.

15. Synthetic Drugs:

15.1 Scope: Synthetic drugs, such as synthetic cannabinoids, substituted cathinones, etc. are generally found on plant materials and/or in paraphernalia. Tests such as thin layer chromatography (TLC), gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS) and gas Chromatography-infrared Spectroscopy (GC-IR) are available to be used to identify the components of these drug-laced plant materials and in/on paraphernalia. Gas chromatography-Infrared spectroscopy is available through the Indianapolis Laboratory.

15.2 Precautions/Limitations:

15.2.1 Synthetic drugs include several different types of substances. Many positional isomers are possible. Analysts shall be prepared to acknowledge the existence of other positional isomers, particularly when the specific isomeric form has not been identified.

15.2.2 Some synthetic drugs convert to other drugs in the injection port and TLC or IR will be needed to verify them (example – 25I-NBOH converts to 2C-I in the GC/MS).

15.2.3 Many are not commercially available and therefore reference materials may not be available.

15.2.4 Finely pulverized material, compressed and/or extracted plant material preparations (i.e. baked goods, residues, etc.) pose difficulties due to the small size of the material, the matrices involved, and potential for complex mixtures.

15.2.5 Synthetic drugs are found in/on paraphernalia similar to those commonly found with Marijuana evidence.

15.2.6 Synthetic cannabinoids are chemically different than traditional “cannabinoids”.

15.2.7 Synthetic cannabinoids and other synthetic drugs do not give reactions to the Duquenois-Levine test for cannabinoids.

15.2.8 Items commonly have multiple drugs present. Additional screening may be necessary to detect other components of the samples.

15.2.9 Substances in synthetic drug mixtures may not resolve sufficiently by Thin Layer Chromatography and therefore TLC may not be a good second test for identification.

15.2.10 Generally the material is not in sufficient quantity or condition for Fourier Transform Infrared Spectroscopy (FTIR) to be possible or practical. If GC-IR is available and practical, it may be used for identification.

15.2.11 GC retention time is generally necessary for identification.

15.3 Related Information:

15.3.1 Appendix 1 – Forms and Worksheets
15.3.2 Appendix 2 – Abbreviations
15.3.3 Appendix 3 – Definitions
15.3.4 Appendix 4 – Drug Unit Reagent Preparation Manual
15.3.5 Other Test Methods
- **15.3.5.1** General Drug Identification
- **15.3.5.2** Weighing Determinations
- **15.3.5.3** Evidence Handling
- **15.3.5.4** Sampling
- **15.3.5.5** TLC
- **15.3.5.6** FTIR
- **15.3.5.7** GC/MS
- **15.3.5.8** GC-IR

15.4 Instruments:

15.4.1 GC/MS, with retention time
15.4.2 FTIR or GC-IR

15.5 Reagents/Materials:

15.5.1 TLC supplies (See TLC Test Method)
15.5.2 GC/MS Supplies (See GC/MS Test Method)
15.5.3 GC-IR Supplies (See GC-IR Test Method)

15.6 Hazards/Safety:

15.6.1 Like all plant materials, moldy plant material presents both health and fire hazards. Wet and moldy plant material generates its own heat and can start a fire if left unattended.

15.6.2 Chemical exposures/inhalation hazards including potential carcinogens may exist.

15.6.3 Some synthetic cannabinoids are more potent than traditional cannabinoids. No known toxicity studies have been performed on humans.

15.7 Reference Materials/Controls/Calibration Checks:

15.7.1 TLC: Appropriate reference materials shall be run with the blanks and samples. (See TLC Test Method.)

15.7.2 GC/MS: Appropriate reference materials shall be used for comparison. (See GC/MS Test Method.)

15.7.3 FTIR: Appropriate reference materials shall be used for comparison. (See FTIR Test Method)

15.7.4 GC-IR: Appropriate reference materials shall be used for comparison. (See GC-IR Test Method)

15.8 Procedures/Instructions:

15.8.1 All suspected synthetic cannabinoid items, except residues, ashes and cigarette butts, shall be weighed.

15.8.2 Items of plant material can be administratively withdrawn if no additional weight thresholds will be met by the analysis of these items. The unexamined evidence shall be weighed. If the plant material can be seen through the outermost packaging, the evidence does not need to be opened and a gross weight including outermost packaging can be taken.

15.8.3 Microscopic Examination: Suspected synthetic drugs are found on a variety of plant materials. All plant materials should be examined microscopically.

15.8.4 Duquenois-Levine Color Test: The Duquenois-Levine Color Test is used as a preliminary test for cannabinoids. Since the synthetic cannabinoids are not true cannabinoids and do not respond to the test, it may help rule out the presence of marijuana. Other synthetic drugs are not known to respond to the Duquenois-Levine test.

15.8.5 Unknown samples and reference materials are routinely dissolved in methanol or chloroform. Other solvents may be suitable.

15.8.6 Thin Layer Chromatography: TLC may be useful to analyze single component samples. There may not be sufficient resolution using general chromatography systems for mixtures.

General TLC systems may help screen synthetic cannabinoid samples for the presence of other drugs that may not be readily apparent when using the normal GC/MS program (See 15.2.8 and TLC Test Method).

15.8.7 Fourier Transform Infrared Spectroscopy: This method gives the most specific structural information available, if and when the sample is in a sufficient quantity to permit the test.

15.8.8 Gas Chromatography/Mass Spectrometry: Generally the synthetic cannabinoids elute at higher temperatures. A general high temperature program may be sufficient, however if no peaks are identified, then a general screening temperature program shall be used. (See 15.2.8, 15.8.5, and GC/MS Test Method).

15.8.9 GC Retention Time: Due to the possibility of multiple positional isomers, GC retention time comparison is necessary when identifying controlled synthetic cannabinoids and their respective isomeric forms. Additionally, GC retention time may be necessary as a second test for identification of the components in a mixture. Routine use is an authorized exception to the GC/MS Test Method.

15.8.10 Gas Chromatography-Infrared Spectroscopy: This is a complementary technique that can distinguish between most positional isomers. It is particularly useful with mixtures or when GC retention time comparisons are not sufficient to determine a specific form or isomer of a drug. (See GC-IR Test Method 10.8.5 for sample criteria.)

15.9 Records:

15.9.1 All weights used to meet or exceed weight limits of a particular criminal charge shall be recorded as net weight.

15.9.2 If macroscopic and/or microscopic botanical characteristics are observed, they shall be recorded in the analysis notes.

15.9.3 Duquenois-Levine: Conclusions as to the solvent blank and the reaction(s) of the unknown sample shall be recorded in the analysis notes.

15.9.4 Thin Layer Chromatography: See TLC Test Method.

15.9.5 Gas Chromatography/Mass Spectrometry: See GC/MS Test Method.

15.9.6 Gas Chromatography Retention Time: See GC/MS Test Method 9.8.4, 9.10.5, and 9.9.5.11.

15.9.7 Gas Chromatography-Infrared Spectroscopy: See GC-IR Test Method.

15.10 Interpretations of Results:

15.10.1 Microscopic examination: If used, see Marijuana Test Method. There are no helpful botanical features associated with synthetic cannabinoids or other synthetic drugs.

15.10.2 Duquenois-Levine: If used, see Color Tests and Marijuana Test Methods. Synthetic cannabinoids and other synthetic drugs do not respond to this test.

15.10.3 Thin Layer Chromatography: Positive indication of the substances present in the unknown sample shall be based on color and location of spots on the plate relative to the reference material(s). This is best used for single component samples. Components in mixtures may not be resolved enough to use this method as a second test. TLC may help rule out the presence of drugs other than synthetic drugs.

15.10.4 Fourier Transform Infrared Spectroscopy – See FTIR Test Method.

15.10.5 Gas Chromatography- Mass Spectrometry: (See GC/MS Test Method 9.10.5). Higher temperature programs are generally sufficient for identification, however some substances may be missed if other screening techniques are not employed (e.g. TLC) to rule out the presence of other drugs. Retention time can be used as a second test for identification when necessary. (See 9.9.5.11)

15.10.6 Gas Chromatography-Infrared Spectroscopy: (See GC-IR Test Method) Higher temperature programs are necessary for most synthetic drugs. As with GC/MS, substances may be missed if other screening techniques are not employed (e.g. TLC, general GC/MS temperature program) to rule out the presence of other drugs.

15.10.7 If multiple controlled synthetics or potential isomers are present in the item, at a minimum, one shall be confirmed. The remaining substances shall be indicated in the analytical notes or on the data (at a minimum).

15.11 Report Writing:

15.11.1 Weights obtained to meet or exceed charges shall be reported as a net weight.

15.11.2 In some cases items of plant material may be administratively withdrawn because no additional weight thresholds have been met.

15.11.2.1 If the evidence is not opened, the report shall use the following or similar verbiage:

Item 001 was not analyzed or opened.

The gross weight of item 001, including outermost packaging, was X gram(s).

15.11.2.2 If the evidence is opened and weighed, the report shall use the following or similar verbiage:

Item 001 was not analyzed.
The net (or gross) weight of item 001 was X gram(s).

15.11.3 If the date of seizure is before the effective date that a drug became controlled, the control status shall be omitted and an additional statement shall be included into the report indicating the date of control.

Example: Item 001 was found to contain AM-2201. AM-2201 was controlled in the State of Indiana as of March 15, 2012. The specific isomer was not determined.

OR

Item 001 indicated the presence of AM-2201. AM-2201 was controlled in the State of Indiana as of March 15, 2012.

If there is reasonable doubt as to the control status of a drug, the control status can be omitted. The analyst shall discuss this with their immediate supervisor.

15.11.4 If the date of seizure is before the effective date that a drug became state controlled, but after the date of federal control, the report shall indicate that it is a federally controlled substance. The date of control in the State of Indiana shall also be included on the report. If the date of seizure is after the date it was controlled in the State of Indiana, the date of federal control is not required to be on the Certificate of Analysis.

15.11.5 If the specific isomeric form has not been determined, the following statement shall be included in the results:

“The specific isomer was not determined.”

If the isomeric form has been determined, this statement shall be omitted. The specific isomer can only be determined by GC retention time if the reference materials for potential positional isomers have been run on the instrument, or if IR spectral data has been obtained.

If the compound is controlled by its structure, the isomer statement may be omitted.

15.11.6 Substances specifically named in the Indiana Criminal Code may be reported as listed in the Indiana Criminal Code.

Example: Item 001 was found to contain JWH-018, a controlled substance. The specific isomer was not determined.

15.11.7 Substances controlled by structure are to be reported by name and a reference to the grouping to which it belongs in the Criminal Code. The report wording shall reflect what is written in the Criminal Code.

Examples:

Item 001 was found to contain JWH-016, a controlled substance structurally derived from 3-(1-naphthoyl)indole.

OR

Item 001 was found to contain JWH-016, a controlled substance. JWH-016 is (a compound) structurally derived from 3-(1-naphthoyl)indole.

15.11.8 If a synthetic drug is controlled because it is substantially similar to a controlled substance, the report shall state the name of the controlled substance that the synthetic drug is substantially similar to.

15.11.9 Substances shall not be reported as “analogs” or “isomers” unless directed by the analyst’s immediate supervisor.

15.11.10 Indications: If a synthetic cannabinoid/drug is indicated, but not identified, the structure based wording and isomer statements may be omitted from the results.

15.11.11 See General Drug Identification.

15.12 References:

15.12.1 Drug Unit Resource Manual – Marijuana

15.12.2 Indiana Criminal Code, IC 35-48-2-4

15.12.3 Indiana Criminal Code , IC 35-31.5-2-321

16. Cocaine:

16.1. Scope: Cocaine is a naturally occurring alkaloid that is extracted from the Erythroxylum coca plant. This test method is intended as a guide for qualitative analysis only.

16.2. Precautions/Limitations:

16.2.1. Cis and trans cinnamoyl cocaines are frequently present in cocaine samples. These are natural products of the coca plant.

16.2.2. Ecgonine, methylecgonine and benzoylecgonine may be present in sample as a result of the purification process, or may be produced by the high temperatures in the GC/MS.

16.2.3. Illicit samples may contain a large variety of substances such as Procaine, Lidocaine, Benzocaine, and/or other drugs.

16.2.4. Cocaine mixtures containing alkaline substances, such as sodium bicarbonate, may convert the form of the Cocaine when water or aqueous solutions are added.

16.2.5. Salt or base form determination is necessary for Federal charges and/or sentencing requirements.

16.2.6. The condition of the sample may prohibit salt form determination.

16.2.7. Cocaine samples are soluble in methanol and chloroform. Chloroform may be preferable due to some samples have been shown to degrade in some methanol solutions.

16.3. Related Information:

16.3.1. Appendix 1 – Forms and Worksheets
16.3.2. Appendix 2 – Abbreviations
16.3.3. Appendix 3 – Definitions
16.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
16.3.5. Other Test Methods
- **16.3.5.1.** General Drug Identification
- **16.3.5.2.** Color (Spot) Tests
- **16.3.5.3.** UV
- **16.3.5.4.** TLC
- **16.3.5.5.** FTIR
- **16.3.5.6.** GC/MS
- **16.3.5.7.** GC-IR
- **16.3.5.8.** Separations and Extractions

16.4. Instruments:

16.4.1. UV
16.4.2. FTIR
16.4.3. GC/MS
16.4.4. GC-IR

16.5. Reagents/Materials:

16.5.1. See Separations and Extraction Test Method
16.5.2. See Color (Spot) Test Reagent Preparation Guide

16.6. Hazards/Safety:

16.6.1. Exposure: numbness of fingers or areas that have been in direct contact with the drug.

16.6.2. Chemical Exposure hazards

16.6.3. See MSDS for Cocaine and related substances.

16.7. Reference Materials/Controls/Calibration Checks:

16.7.1. Appropriate Reference Materials for Cocaine, related materials, excipients and diluents.

16.8. Procedures/Instructions:

16.8.1. See General Drug Identification Test Method.

16.8.2. Color (Spot) Tests: The recommended color tests for Cocaine are the Cobalt Thiocyanate or Scott Tests.

16.8.3. UV – generally Cocaine type samples are analyzed in 0.5 N H_2SO_4

16.8.4. TLC Recommended Systems

16.8.4.1. General TLC solvent systems:
MeOH :NH_4OH (100:1.5)
$CHCl_3$:MeOH:HOAc (75:20:5)

16.8.5. Extraction: Cocaine is very soluble in $CHCl_3$, Pet. Ether and Methanol. It has a low solubility in water in the base form. Generally, Cocaine is extracted with organic solvents from aqueous alkaline solutions. The following are common extractions used for purifying street samples containing Cocaine:

16.8.5.1. Cocaine Base extraction: Pet. Ether dry extract, or dissolve in Pet. Ether and wash with distilled water.

16.8.5.2. General Cocaine Extraction: Pet. Ether or $CHCl_3$ from base (0.45N NaOH)

16.8.5.3. Other complex mixtures with Cocaine, See Drug Unit Resource Manual(s).

16.8.6. Gas Chromatography/Mass Spectrometry, Gas Chromatography-Infrared Spectroscopy*, and or FTIR can be used for confirmation. (*Note: salt forms cannot be determined by using GC-IR)

16.9. Records:

16.9.1. See General Drug Identification
16.9.2. See Other Test Methods

16.10. Interpretations of Results:

16.10.1. Color Tests

16.10.1.1. Cobalt Thiocyanate = Cocaine HCl, Procaine, Lidocaine, Benzocaine forms a blue precipitate; Cocaine Base turns a slow blue. There are many other substances that react similarly to Cocaine with this test.

16.10.1.2. Scott's Test = Cocaine turns blue in the first step. The blue should disappear with addition of HCl to give a pink solution. The mixture should turn blue again when $CHCl_3$ is added and the mixture shaken.

16.10.2. UV in acid (0.5N H_2SO_4) 233, 275 nm for Cocaine. Shifts occur when mixed with other substances. The degree, direction and shape of the shift may indicate the identity of the interfering substance.

16.10.3. Thin Layer Chromatography/Over-sprays:

16.10.3.1. Ninhydrin – turns Procaine and Benzocaine pink
16.10.3.2. p-DMAB – turns Procaine and Benzocaine yellow
16.10.3.3. Iodoplatinate
16.10.3.4. Potassium Permanganate ($KMnO_4$)

16.10.4. FTIR: See FTIR Test Method and Reference Material Test Method.

16.10.5. GC/MS: See GC/MS Test Method.

16.10.6. GC-IR: See GC-IR Test Method.

16.11. Report Writing:

16.11.1. The base form shall only be reported if requested by the Prosecutor or with supervisory approval.

16.12. References:

16.12.1. Analytical Profiles of Cocaine, Local Anesthetics and Common Diluents Found with Cocaine, CND Analytical, Inc. 1990

16.12.2. Cocaine, Marijuana, Designer Drugs: Chemistry, Pharmacology and Behavior, K. Redda, C. Walker, G. Barnett, CRC Press, 2000.

16.12.3. The Analysis of Controlled Substances, Cole, Michael D., Wiley, 2003

16.12.4. Drug Unit Cocaine Resource Manual

17. Tryptamines/Indoles (General):

17.1. Scope: This Test Method covers substances that contain the indole nucleus and may be classified as hallucinogens. This group includes drugs such as Tryptamines, Psilocybic Mushrooms and Lysergic Acid Diethylamide (LSD). Psilocybic mushrooms and LSD will be covered in detail in separate Test Methods due to their complex analytical requirements.

17.2. Precautions/Limitations:

17.2.1. Hallucinogenic
17.2.2. Typically small dosages, but potent.
17.2.3. The media in which the drug resides usually comprises the majority of the weight of the exhibit.

17.3. Related Information:

17.3.1. Appendix 1 – Forms and Worksheets
17.3.2. Appendix 2 – Abbreviations
17.3.3. Appendix 3 – Definitions
17.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
17.3.5. Other Test Methods
17.3.5.1. General Drug Identification
17.3.5.2. Color Tests
17.3.5.3. UV
17.3.5.4. TLC
17.3.5.5. FTIR
17.3.5.6. GC/MS
17.3.5.7. GC-IR
17.3.5.8. Separation and Extractions Procedures

17.4. Instruments:

17.4.1. UV
17.4.2. FTIR
17.4.3. GC/MS
17.4.4. GC-IR

17.5. Reagents/Materials:

17.5.1. See Color (Spot) Tests Test Method
17.5.2. See Thin Layer Chromatography Test Method
17.5.3. Methanol (MeOH)
17.5.4. Chloroform ($CHCl_3$)

17.6. Hazards/Safety: Exposure - skin absorption of hallucinogenic drugs.

17.7. Reference Materials/Controls/Calibration Checks:

17.7.1 Reference materials as appropriate.

17.8. Procedures/Instructions:

17.8.1. Extraction: See Separation and Extractions Test Method and Reference Materials.
17.8.2. Color (Spot) Tests: Marquis, p-DMAB, Mecke's
17.8.3. UV (in acid)
17.8.4. TLC Systems: MeOH :NH_4OH (100:1.5)
17.8.5. FTIR – extracted
17.8.6. GC/MS – extracted
17.8.7. GC-IR - extracted

17.9. Records: See Other Test Methods.

17.10. Interpretations of Results:

17.10.1. Color Tests

Marquis – strong blues, Substituted tryptamines – some olive green

p-DMAB – purple with LSD, grey/violet with indole alkaloids, also various pink colors are possible.

Mecke's – strong reactions – blues, purples, grey-black

17.10.2. UV – Generally strong UV absorbers with absorbance patterns that are characteristic of the group.

17.10.3. TLC – See TLC Test Method

17.10.4. FTIR – See FTIR Test Method

17.10.5. GC/MS - See GC/MS Test Method

17.10.6. GC-IR – See GC-IR Test Method

17.11. Report Writing: See General Drug Identification.

17.12. References:

17.12.1. Drug Unit Resource Manual

17.12.2. Tryptamines Volume 1: Synthesis, Analog Synthesis and Precursor Synthesis, Clandestine Laboratory Investigating Chemists, 2001

17.12.3. Tryptamines Volume 2: Analytical Data and Natural Product Synthesis, Clandestine Laboratory Investigating Chemists, 2001

17.12.4. Analytical Profiles for Five "Designer" Tryptamines, Spratley, et. al. (US Department of Justice, DEA), Microgram Journal, Vol. 1, Jan-Jun 2003.

18. Lysergic Acid Diethylamide (LSD):

18.1. Scope: LSD is a synthetic hallucinogen commonly found in liquid form, on blotter paper, tablets (microdots), windowpanes and sugar cubes. This Test Method is intended to outline the procedures for identification of LSD that is usually present in very small quantities and/or concentrations.

18.2. Precautions/Limitations:

18.2.1. A structural isomer exists [Lysergic Acid Methyl Propylamide (LAMPA)], which produces similar GC/MS spectral data, but can be differentiated by Thin Layer Chromatography and GC retention time.

18.2.2. GC-IR analysis may be used; however, limited concentrations of sample material may not produce IR data of sufficient quality to permit identification.

18.2.3. LSD also has a stereoisomer, Iso-LSD, which has different physical and chemical properties than LSD. It can be easily separated from LSD by using Thin Layer Chromatography. However, a reference material may not be available.

18.2.4. Small amount of drug per dosage unit.

18.2.5. Presence of dyes and/or other complex media can interfere with analysis.

18.2.6. LSD has an affinity for filter papers, and the resulting extraction yields will be very low, if anything at all.

18.3. Related Information:

18.3.1. Appendix 1 – Forms and Worksheets
18.3.2. Appendix 2 – Abbreviations
18.3.3. Appendix 3 – Definitions
18.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
18.3.5. Other Test Methods
- **18.3.5.1.** General Drug Identification
- **18.3.5.2.** Color Tests
- **18.3.5.3.** UV
- **18.3.5.4.** TLC
- **18.3.5.5.** FTIR
- **18.3.5.6.** GC/MS
- **18.3.5.7.** GC-IR
- **18.3.5.8.** Separations

18.4. Instruments:

18.4.1. UV
18.4.2. UV light box or other UV light source
18.4.3. FTIR, possibly, but not common due to insufficient sample size
18.4.4. GC/MS
18.4.5. GC-IR, possibly, if enough sample exists.

18.5. Reagents/Materials:

18.5.1. p-DMAB Color Test Reagent
18.5.2. Concentrated Hydrochloric acid (HCl)
18.5.3. Methanol
18.5.4. Chloroform
18.5.5. Extraction chemicals
18.5.6. TLC system chemicals
18.5.7. Chemical over-sprays
18.5.8. Laboratory glassware

18.6. Hazards/Safety:

18.6.1. Exposure – through skin contact, solvent exposure.
18.6.2. See MSDS for drugs and chemicals used in analysis.

18.7. Reference Materials/Controls/Calibration Checks:

18.7.1. Reference Materials of LSD and LAMPA.

18.8. Procedures/Instructions:

18.8.1. See General Drug Identification

18.8.2. Suggested Extractions:

18.8.2.1. Methanol, or

18.8.2.2. Filter Methanol through a glass pipette (with a glass wool plug and filled with alumina), or

18.8.2.3. Sugar Cube Extraction: In a separatory funnel containing a crushed sugar cube, add 10-15 ml of a 1% tartaric acid solution.

Add ~20 ml $CHCl_3$ and shake. Drain. Repeat one time.

Make solution basic with a NaOH pellet.

Add ~30ml $CHCl_3$ and shake for several minutes.

Drain $CHCl_3$ into a ~50 ml beaker using NO FILTER PAPER.

Evaporate to dryness.

Add two drops of MeOH for use in TLC or GC/MS. GC-IR may also be used, if enough sample exists and this technique is available.

18.8.2.4. Windowpanes: Cut or crush the windowpane. Soak the crushed windowpane in MeOH for an extended time period; or

Soak the crushed windowpane in 0.45 N NaOH for several hours to dissolve the windowpane. Extract with $CHCl_3$ and evaporate to dryness.

18.8.3. Color Test – p-DMAB

18.8.4. UV in Methanol

18.8.5. TLC Systems (Suggested):

18.8.5.1. Acetone

18.8.5.2. Acetone: NH_4OH sat'd $CHCl_3$ (9:1)

18.8.5.3. Over-spray with p-DMAB. It may be necessary to heat the plate to get good results with the overspray.

18.8.6. TLC with degradation (optional)

18.8.6.1. Prepare TLC plates by spotting samples and reference materials.

18.8.6.2. Expose to short wave UV light for approximately 30 minutes.

18.8.6.3. Place the plate in the TLC tank and develop.

18.8.6.4. After plate is removed and dried, look at the plate under short and long wave UV light. Mark spots with pencil.

18.8.6.5. Spray the plate with p-DMAB.

18.8.6.6. Observe and compare degradation spots in samples and reference materials.

18.8.7. GC/MS: Temperature programs from approximately 240 to 280 degrees Celsius.

18.8.8. GC-IR: Similar temperature programs used in GC/MS may be appropriate.

18.9. Records: See General Drug Identification Test Method

18.10. Interpretations of Results:

18.10.1. Color Test p-DMAB Positive = purple with LSD

18.10.2. UV in Methanol = approximately 310nm

18.10.3. UV light box: Both LSD and LAMPA fluoresce blue under long wave UV light

18.10.4. TLC - LSD and LAMPA should separate and turn purple/blue with p-DMAB over-spray.

18.10.5. TLC with degradation: compare sample degradation spot locations and reactions to over-spray with the degradation spots of the reference material.

18.10.6. GC/MS: See GC/MS Test Method. Care should be taken to evaluate the spectrum closely when comparing LSD and LAMPA.

18.10.7. GC-IR: See GC-IR Test Method. LSD and LAMPA can be clearly distinguished using this method. Concentration and chromatography quality may not be sufficient for identification.

18.11. Report Writing: See General Drug Identification

18.12. References:

18.12.1. Lysergic Acid Amide Workshop, Rosenthal, J. Midwestern Association of Forensic Scientists (MAFS) , Oct 1998

18.12.2. LSD Analysis, Robison, Mary; 1983

18.12.3. Differentiation of LSD and LAMPA, Kebabjian, Dennis; Microgram, Vol. VIII, No. 4 (April, 1975) pp 53-54

18.12.4. A Technique for the Infrared Identification of LSD, Clodfelter, Ronald; Microgram, Vol. VIII, No. 9, (Sept 1975) pp 137-138.

18.12.5. Micro-Infrared Analysis of LSD, Morgan and Francois, Microgram, Vol. IX, No. 9 (Sept 1976) pp 130-135.

18.12.6. Basic Training Program for Forensic Drug Chemists, US Department of Justice Bureau of Narcotic and Dangerous Drugs; Canaff, May 1972.

19. Psilocybin Mushrooms:

19.1. Scope: Mushrooms encountered in routine case work often contain Psilocyn and/or Psilocybin. The mushroom (genus *Psilocybe*) itself is not controlled, but rather the hallucinogens found within it.

19.2. Precautions/Limitations:

19.2.1. Extraction is necessary for identification. Mushrooms contain large amounts of alkaloids, fats and sugars that complicate analysis and must be removed.

19.2.2. Psilocybin cannot be identified by GC/MS or GC-IR alone, since it breaks down into Psilocyn in the injection port. Thin Layer Chromatography is required for information to support identification. If Psilocybin identification is needed, derivatization may be necessary.

19.2.3. Psilocybin is the phosphorylated ester of Psilocyn and easily converts to Psilocyn with heat and during extraction with acid or alkaline solutions.

19.2.4. TLC must be performed on a methanol extract prior to further extraction to determine the presence of Psilocyn or Psilocybin, or both.

19.2.5. FTIR is not generally performed due to insufficient sample size and extraction is not sufficient to isolate Psilocyn from Psilocybin.

19.2.6. Acetylpsilocyn converts to Psilocyn in the injection port and derivatization may be required.

19.3. Related Information:

19.3.1. Appendix 1 – Forms and Worksheets
19.3.2. Appendix 2 – Abbreviations
19.3.3. Appendix 3 – Definitions
19.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
19.3.5. Other Test Methods
- **19.3.5.1.** General Drug Identification
- **19.3.5.2.** Color Tests
- **19.3.5.3.** UV
- **19.3.5.4.** TLC
- **19.3.5.5.** Separations
- **19.3.5.6.** GC/MS
- **19.3.5.7.** GC-IR

19.4. Instruments:
19.4.1. UV
19.4.2. GC/MS
19.4.3. GC-IR

19.5. Reagents/Materials:

19.5.1. Color (Spot) Test Reagents
19.5.2. pH paper
19.5.3. TLC Solvent Systems and supplies
19.5.4. Chemical over-sprays/visualization reagents
19.5.5. Methanol
19.5.6. Acetone
19.5.7. $CHCl_3$
19.5.8. Acetic Acid
19.5.9. Ammonium hydroxide
19.5.10. Extraction chemicals

19.6. Hazards/Safety: See MSDS.

19.6.1. Exposure to hallucinogenic drugs
19.6.2. Exposure to hazardous chemicals

19.7. Reference Materials/Controls/Calibration Checks:

19.7.1. Psilocybin and Psilocyn Reference Materials.

19.8. Procedures/Instructions:

19.8.1. Visual examination of mushroom, note presence of blue bruises on stems and odor, if present.

19.8.2. Color Tests (i.e. p-DMAB or Weber), if desired, direct on a portion of the mushroom or on an extract.

19.8.3. Sample Preparation:

Optional: Pulverize the mushroom.

Soak in Methanol.

Optional: Heat MeOH/Mushroom mixture (@40 degrees Celsius max) for two hours.

Pour off MeOH into a clean beaker and filter. Repeat MeOH soak, if desired.

Optional Step: Add 10ml Acetone, and place in freezer for 30 minutes to freeze out the fats. Filter.

Evaporate to dryness in a beaker without heat.

19.8.4. Ultraviolet Spectroscopy in MeOH; can be run before or after extraction.

19.8.5. ***Reconstitute in MeOH to run TLC***

19.8.6. Recommended TLC Systems:

19.8.6.1. MeOH: NH_4OH (100:1.5)
19.8.6.2. $CHCl_3$:MeOH:HOAc (75:20:5)
19.8.6.3. n-butanol:dH_2O:HOAc (2:1:1)
19.8.6.4. Overspray with acidified p-DMAB

19.8.7. Recommended Extraction for GC/MS and GC-IR:

Dissolve sample (from MeOH extract) with 1% – 5% acetic acid and pour into separatory funnel.

Rinse sample beaker with 1% – 5% acetic acid and pour into the separatory funnel.

Rinse beaker again with $CHCl_3$ and pour into the same separatory funnel.

Add more $CHCl_3$ and extract.

Discard the $CHCl_3$.

Make the aqueous layer basic with NH_4OH, extract with $CHCl_3$.

Evaporate to dryness.

Reconstitute in MeOH and run on GC/MS.

19.8.8. GC/MS - See GC/MS Test Method.

19.8.9. GC-IR: See GC-IR Test Method.

19.9. Records: See General Drug Identification.

19.10. Interpretations of Results:

19.10.1. Visual Examination: blue-grey bruising is indicative of oxidation of indole-containing compounds.

19.10.2. Odors associated with Psilocybic Mushrooms are generally unpleasant, but are characteristic of these types of mushrooms.

19.10.3. Color Tests:

19.10.3.1. p-DMAB (Ehrlich's) = purple-black

19.10.3.2. Weber's = Fast Blue B = red, addition of HCl = blue

19.10.3.3. Mecke's – green color

19.10.4. UV (acid) Psilocyn 266, 283,292 nm; Psilocybin 268 nm, w/shoulder at 287 nm

19.10.5. UV (Base) Psilocyn 270, 293 nm; Psilocybin 269, 282, 292 nm.

19.10.6. UV (methanol) Psilocyn; Psilocybin 267, 280, 290 nm.

19.10.7. TLC: See TLC Test Method (7.10). It is essential that separation occurs between Psilocyn and Psilocybin reference materials.

19.10.8. GC/MS without derivatization will identify only Psilocyn since Psilocybin breaks down in the injection port of the GC.

Since Psilocybin converts to Psilocyn, TLC is essential when determining which substances are present. If no Psilocybin is present on TLC, it can be concluded that the GC/MS is that of Psilocyn and only Psilocyn.

If TLC reveals the presence of both Psilocyn and Psilocybin, the resulting GC/MS spectrum can be concluded to be some combination of both Psilocyn and converted Psilocybin.

If TLC reveals the presence of only Psilocybin, the resulting GC/MS will result in the spectrum of Psilocyn, but is in reality converted Psilocybin. The combinations of TLC and GC/MS results are sufficient to make the conclusion that the sample contained Psilocybin if the TLC was performed prior to the acid/base extraction procedures.

19.10.9. GC-IR would not be able to differentiate between Psilocyn and Psilocybin. The same breakdown issues that occur with GC/MS also exist with this technique.

19.11. Report Writing:

19.11.1. If Psilocyn is the only substance indicated on TLC and identified by GC/MS (or GC-IR), it shall be reported as "Psilocyn".

19.11.2. If Psilocybin is the only substance identified on TLC and supported by GC/MS (or GC-IR) spectral data for Psilocyn, it may be identified and reported as "Psilocybin".

19.11.3. If Psilocyn and Psilocybin are indicated on TLC and Psilocyn was identified by GC/MS (or GC-IR), the item shall be reported using the following verbiage: "was found to contain Psilocyn, a controlled substance. Examination also indicated the presence of Psilocybin, a controlled substance."

19.12. References:

19.12.1. Isolation and Identification of Psilocybin and Psilocin, M.A. Bonin (US Army Criminal Investigation Laboratory, Fort Gordon, GA), Microgram Vol. XVI, No. 6, June 1983

19.12.2. Drug Unit Resource Manual

19.12.2.1. Hallucinogenic Mushrooms, Oliveria and Medeiros de Silva (translated by Morris Grodsky); Microgram, Vol XI, No.2 (February , 1978)

19.12.2.2. The Identification of Psilocyn and Psilocybin in Mushrooms Using High Resolution Gas Chromatography/Mass Spectrometry, Timmons, James E. (Arizona Department of Public Safety, Phoenix, AZ), Microgram, Vol. XVII, No. 2, February 1984.

19.12.2.3. Identification of Psilocybin in Mushrooms, Miller Daniel S. (Florida Department of Law Enforcement)

19.12.2.4. The Assay of Psilocybe Mushrooms for Hallucinogens, The Drug Chromatographer, Volume 1992.2, Bulletin 244 Alltech Applied Science Labs.

19.12.2.5. An Aqueous-Organic Extraction Method for the Isolation and Identification of Psilocin from Hallucinogenic Mushrooms, Casale, John F., Journal of Forensic Sciences, Vol 30, No. 1, Jan 1985, pp 247-250.

19.12.2.6. Psilocybin Mushroom Workshop, Penabraker, Scott; Midwestern Association of Forensic Scientists (MAFS), Oct 1998.

19.12.3. Tryptamines Volume 1: Synthesis, Analog Synthesis and Precursor Synthesis, Clandestine Laboratory Investigating Chemists, 2001

19.12.4. Tryptamines Volume 2: Analytical Data and Natural Product Synthesis, Clandestine Laboratory Investigating Chemists, 2001

19.12.4.1. Quantitative Analysis of Psilocybin and Psilocin in *Psilocybe Baecystis* (Singer and Smith) by High Performance Liquid

Chromatography and by Thin Layer Chromatography, Beug, M. and Bigwood, J., Journal of Chromatography, 207 (1981) P 370-385

19.12.4.2. Botanical and Chemical Characterisation of Forensic Mushroom Specimen of the Genus *Psilocybe*, Heim, Genest, Hughes, and Belec; Journal of Forensic Science Society, Vol 6, No. 4, 1966

19.12.4.3. Weber Test; Garrett, Allen; Clemens, Steven and Gaskill, James. Weber State College, Laboratory of Criminalistics, Ogden, Utah.

19.12.4.4. Blueing on *Conocybe, Psilocybe* and a *Stropharia* and the Detection of Psilocybin., Benedict, Tyler and Watling; Lloydia, Vol. 30, No.2 , June 1967

19.12.5. TiHKAL The Continuation, Shulgin, Alexander and Shulgin, Ann; Transform Press, Berkeley, CA, 1997

19.12.6. Analysis and Characterization of Psilocybin and Psilocyn Using Liquid Chromatography Electrospray Ionization Mass Spectrometry (LC ESI MS) with Collision-Induced-Dissociation (CID) and Source-Induced-Dissociation (SID), Rodriguez, S. (US Dept. Of Justice DEA, Vista, CA), Microgram Journal, Vol 3, No. 34, July- Dec 2005.

20. Khat

20.1. Scope: *Catha Edulis* (Khat) is a plant native to east Africa and southern Arabia that contains two naturally occurring central nervous system (CNS) stimulants, Cathine and Cathinone. Cathinone, the primary active component that is structurally related to amphetamine. Cathine (d-norpseudoephedrine) is related to Pseudoephedrine**.**

20.2. Precautions/Limitations:

20.2.1. Cathinone levels are highest in freshly cut khat plants. Once cut, levels of Cathinone start to decline.

Research indicates enzyme action in the plant material causes the Cathinone (Schedule I) to break down to Cathine (Schedule IV). When the plant material is in a dried state or the Cathinone and Cathine have been removed from the leaves, the enzyme action appears to be slowed down significantly.

It is recommended that the plant material be refrigerated (or frozen if it is to be in storage for a period of time) to reduce the rate of degradation of the Cathinone.

20.2.2. Needs to be carefully extracted to avoid converting Cathinone to Cathine during extraction.

20.2.3. The botanical identification of the Khat plant is beyond the scope of the ISP Drug Unit analysis.

20.3. Related Information:

20.3.1. Appendix 1 – Forms and Worksheets
20.3.2. Appendix 2 – Abbreviations
20.3.3. Appendix 3 – Definitions
20.3.4. Appendix 4 – Drug Unit Reagent Preparation Manual
20.3.5. Other Test Methods
- **20.3.5.1.** Color (Spot) Tests
- **20.3.5.2.** UV
- **20.3.5.3.** TLC
- **20.3.5.4.** FTIR
- **20.3.5.5.** GC/MS
- **20.3.5.6.** GC-IR
- **20.3.5.7.** Separation and Extraction
- **20.3.5.8.** General Drug Identification

20.4. Instruments:

20.4.1. UV
20.4.2. FTIR
20.4.3. GC/MS
20.4.4. GC-IR

20.5. Reagents/Materials: See Other Test Methods

20.6. Hazards/Safety: See MSDS for Cathinone, and Cathine

20.7. Reference Materials/Controls/Calibration Checks:

20.7.1. Reference Materials for Cathinone, and Cathine.

20.8. Procedures/Instructions:

20.8.1. Extraction:
20.8.1.1. Weigh out at least 5 g of plant material (do not crush).
20.8.1.2. Soak in MeOH (enough to cover plant material) for about 30 min.
20.8.1.3. Filter and evaporate to dryness.
20.8.1.4. Dissolve residue in 0.02 N H_2SO_4.
20.8.1.5. Wash with $CHCl_3$.
20.8.1.6. Make aqueous layer basic (pH 8 – 9) with sat. $NaHCO_3/H_2O$.
20.8.1.7. Extract with $CHCl_3$ or CH_2Cl_2.
20.8.1.8. Evaporate down to use for TLC and GC/MS (**DO NOT** evaporate to dryness as oxidation may occur or substances may evaporate – run in $CHCl_3$).

20.8.2. UV – See UV Test Method
20.8.3. TLC – See TLC Test Method
20.8.4. FTIR – See FTIR Test Method
20.8.5. GC/MS – See GC/MS Test Method
20.8.6. GC-IR – See GC-IR Test Method

20.9. Records: See General Drug Identification Test Method.

20.10. Interpretations of Results: The results of the analysis would conclude the presence of Cathinone and/or Cathine. While these results would indicate that the material is consistent with the Khat plant, the botanical identification of the Khat plant is beyond the scope of the ISP Drug Unit analysis.

20.11. Report Writing: See General Drug Identification Reporting.

20.12. References:

20.12.1. Khat Fact Sheet (December 1992), US Department of Justice, Drug Enforcement Administration, Microgram, Vol. XXVI, No. 3 March 1993

20.12.2. The Identification of Cathinone and Methcathinone, Dal Cason, Terry A. (DEA Central Laboratory, Chicago, IL), Microgram, Vol. XXV, No. 12, December 1992.

20.12.3. Drugs and Chemicals of Concern: Khat., Office of Diversion Control Information and Legal Resources, June 2009

20.12.4. The Identification of Cathinone in Khat (Catha Edulis): A Time Study, Lee, M.M. Journal of Forensic Sciences, Vol. 40, No.1, January 1995, pp116-121.

20.12.5. ISP Khat Extraction Validation of 20.12.4

21. Methoxyamphetamines:

21.1 Scope: Methoxyamphetamines are frequently call “designer drugs” and are closely related to the indoles and phenethylamines. These compounds may be synthetic, semi-synthetic or naturally occurring. Most commonly they tend to be found in club drugs and sold as hallucinogens.

21.2 Precautions/Limitations:

21.2.1 Hallucinogenic in nature.

21.2.2 Frequently found with other controlled substances and a variety of adulterants.

21.2.3 Many regioisomeric forms exist and may be difficult to differentiate.

21.2.4 Peyote – buttons must be dry and finely ground before extraction in order to isolate mescaline.

21.3 Related Information:

21.3.1 Appendix 1 – Forms and Worksheets
21.3.2 Appendix 2 – Abbreviations
21.3.3 Appendix 3 – Definitions
21.3.4 Appendix 4 – Drug Unit Reagent Preparation Manual
21.3.5 Other Test Methods
- **21.3.5.1** General Drug Identification
- **21.3.5.2** Color Tests
- **21.3.5.3** UV
- **21.3.5.4** FTIR
- **21.3.5.5** GC/MS
- **21.3.5.6** GC-IR
- **21.3.5.7** Separations
- **21.3.5.8** Clandestine Laboratory Sample Analysis.

21.4 Instruments:

21.4.1 UV
21.4.2 FTIR
21.4.3 GC/MS
21.4.4 GC-IR

21.5 Reagents/Materials:

21.5.1 See Color (Spot) Tests Test Method
21.5.2 See General Test Methods

21.6 Hazards/Safety: Chemical Exposure – See MSDS

21.7 Reference Materials/Controls/Calibration Checks:

21.7.1 Appropriate Reference Materials for drugs of interest.

21.8 Procedures/Instructions:

21.8.1 Extractions: Generally organic solvents from aqueous alkaline solutions are used and are the same as most Phenethylamines. Some may require special considerations and procedures. These may require HCl fumes to keep from evaporating.

21.8.1.1 Peyote (Mescaline): Dry and Crush peyote buttons.
#1: Soak in MeOH.

Or

#2: Mix approximately 0.5 g sample with 0.1N HCl, make basic with 2.0N NaOH, extract with hexanes. HCl fume. Let evaporate. Run on GC/MS in methanol.

Or

#3: Add 2.0N NaOH to approximately 0.5g sample and extract with $CHCl_3$. Run on GC/MS in $CHCl_3$.

21.8.2 General analytical procedures are sufficient.
21.8.2.1 Color Tests
21.8.2.2 UV in acid (0.5N H_2SO_4)
21.8.2.3 TLC systems:
21.8.2.3.1 MeOH:NH_4OH (100:1.5)
21.8.2.3.2 $CHCl_3$:MeOH:HOAc (75:20:5)
21.8.2.4 FTIR
21.8.2.5 GC/MS
21.8.2.6 GC-IR

21.9 Records: See Other Test Methods

21.10 Interpretations of Results:

21.10.1 Color Tests – generally strong Marquis and Mecke's reactions, ranging from intense blues to grey and black. Some pink and purples are possible as well.

MDMA
Marquis: intense purple to black
Mecke's: fast and intense yellow-green to dark blue to black

Peyote (mescaline):
Marquis: orange
Mecke's: orange-brown

21.10.2 UV in acid: Generally one or two intense peaks for most methoxyamphetamines.

21.10.2.1 Peyote (mescaline) 268 nm

21.10.3 TLC – See TLC Test Method; General Drug Identification

21.10.4 FTIR – See FTIR Test Method, General Drug Identification

21.10.5 GC/MS – See GC/MS Test Method, General Drug Identification

21.10.6 GC-IR – See GC-IR Test Method, General Drug Identification

21.10.7 Peyote (Mescaline): Results of the analysis would conclude the presence of Mescaline. While these results would indicate that the material is consistent with the Peyote Cactus, or Peyote Buttons, the botanical identification of Peyote is beyond the scope of the ISP Drug Unit analysis.

21.11 Report Writing:

21.11.1 See General Drug Identification.

21.11.2 Peyote: See 19.10.7. Results shall be reported as "found to contain Mescaline, a controlled substance", if appropriate.

21.12 References:

21.12.1 Drug Unit Resource Manual

21.12.1.1 Extraction of Mescaline from Peyote, Maloney, David (Jefferson County Sheriff's Office, Golden, Colorado), Microgram, Vol. XXXIV, No. 8, (August 2001).

21.12.1.2 Extraction of Mescaline from Peyote and Subsequent Instrumental Analysis, Barbara, John (State of Tennessee Forensic Laboratory, Knoxville, TN), Microgram, Vol. VIII, No. 12 (December, 1975) p 182-187

21.12.1.3 Extraction of Mescaline from Peyote Buttons, DalCason, Terry A., Microgram, Vol. VI, No.3 (March, 1973) p 43

21.12.1.4 Peyote: Interpretation under Federal Law, Drug Enforcement, (Summer 1975) p 40-41.

21.12.1.5 Isolation and Identification of Drugs, Clarke, E.G.C., Vol. 1 and II.

21.12.1.6 The Identification of Methoxyamphetamine, Methoxy-N-Methylamphetamine and Methylenedioxymethamphetamine, Bailey, K., Legault, D., and Verner, D. (Drug Research Laboratories Health Protection Branch, Ottawa, Canada)

21.12.1.7 Methods of Differentiation for Regioisomeric 2,3- and 3,4-Methylenedioxyphenalkylamines by Liquid Chromatography and Mass Spectrometry, Clark, C. Randall, Noggle, F. Taylor, Holston, Pamela L., and DeRuiter, Jack (Auburn University, Auburn, Alabama), Microgram, Vol. XXXI, No. 9, September 1998.

21.12.2 PiHKAL: A Chemical Love Story, Shulgin, Alexander and Shulgin, Ann, Transform Press, 1991.

21.12.3 A Discussion of 2C-I and Acetylated 2C-T-7, Shanks, Kathy; Koresch, Sandra and Oehldrich, James (Wisconsin State Crime Laboratory Milwaukee, WI)

21.12.4 The Identification of 2,5-Dimethoxy-4-(N)-Propylthiophenenethylamine (2C-T-7), Zimmerman, Michelle M.(Wisconsin State Crime Laboratory, Wausau, WI),Microgram, Vol. XXXIV, No. 7,July 2001.

21.12.5 Analytical Profiles of 4-Bromo-2,5-Dimethoxyphenethylamine (“Nexus”) and Related Precursor Chemicals, Noggle, DeRuiter, and Clark (Alabama Department of Forensic Sciences, Auburn, AL), Microgram, Vol. XXVII, No. 10, October 1994.

22. Phenethylamines:

22.1 Scope: Phenethylamines are central nervous system stimulants and appetite suppressants. Some of the more commonly analyzed substances within this group are Amphetamine, Methamphetamine, Phentermine, Phendimetrazine, Methcathinone, Ephedrine, Pseudoephedrine, and Methylphenidate.

22.2 Precautions/Limitations:

22.2.1 Phenethylamines are typically mixed with a variety of adulterants, diluents, impurities and/or precursors.

22.2.2 Generally phenethylamines are soluble in methanol.

22.2.3 Alkaline extracts of these types of samples may be volatile and are prone to loss if not converted to a stable salt form.

22.2.4 Pharmaceutical preparations may contain Phenethylamines that are contained within resin beads or time release formulations that must be crushed prior to analysis.

22.2.5 Members of this drug grouping are typically very small molecules, which can make GC/MS analysis difficult. Care must be taken when making comparisons due to the limited spectral information available. FTIR may be a better method of confirmation, if the sample quantity permits.

22.2.6 Gas chromatography of salt forms is usually poor. It is advisable to run these in their free base form.

22.3 Related Information:

22.3.1 Appendix 1 – Forms and Worksheets
22.3.2 Appendix 2 – Abbreviations
22.3.3 Appendix 3 – Definitions
22.3.4 Appendix 4 – Drug Unit Reagent Preparation Manual
22.3.5 Other Test Methods
- **22.3.5.1** Clandestine Laboratory Sample Analysis
- **22.3.5.2** Methoxyamphetamines
- **22.3.5.3** Color (Spot) Tests
- **22.3.5.4** UV
- **22.3.5.5** FTIR
- **22.3.5.6** GC/MS

22.3.5.7 GC-IR
22.3.5.8 Separations

22.4 Instruments:

22.4.1 UV
22.4.2 FTIR
22.4.3 GC/MS
22.4.4 GC-IR

22.5 Reagents/Materials:

22.5.1 See General Drug Identification Test Method
22.5.2 See Separations Test Method

22.6 Hazards/Safety:

22.6.1 Chemical Exposure – See MSDS for individual drug hazards.

22.7 Reference Materials/Controls/Calibration Checks:

22.7.1 Appropriate Reference Materials of drug of interest, common excipients and diluents.

22.8 Procedures/Instructions:

22.8.1 Extraction from aqueous alkaline solutions with organic solvents is routinely necessary to obtain good results. Generally petroleum ether or $CHCl_3$ from 0.45N NaOH works well. HCl fumes may be needed to stabilize the drug, depending on the type of analysis to be performed.

22.8.2 Spot Tests – Marquis, Mecke's and Sodium Nitroprusside.

22.8.3 UV in acid (0.5N H_2SO_4)

22.8.4 Thin Layer Chromatography – general drug systems. (See 22.10.3)

22.8.5 FTIR- using ATR or transmittance. Extraction is usually necessary.

22.8.6 GC/MS – general temperature programs with low starting temperatures are sufficient. The addition of sodium bicarbonate ($NaHCO_3$) to a methanolic extraction of phenethylamines improves chromatographic response.

22.8.7 GC-IR – general temperature programs similar to those used in GC/MS are sufficient. The addition of sodium bicarbonate does not improve chromatography with this technique. Full extraction is needed.

22.9 Records: See General Drug Identification Test Method

22.10 Interpretations of Results:

22.10.1 Spot Tests

22.10.1.1 Marquis turns Amphetamine-like substances orange → brown.

22.10.1.2 Phendimetrazine, Phenmetrazine, Ephedrine, Pseudoephedrine, and Propylhexedrine do not give an orange on the Marquis test.

22.10.1.3 Sodium Nitroprusside turns secondary amines, such as Methamphetamine, blue.

22.10.2 UV: Phenethylamines generally give a triplet UV Spectrum in acid (0.5N H_2SO_4) with maxima approximately 251, 257, and 263 nm (Amphetamine).

22.10.2.1 Propylhexedrine gives no UV spectrum.

22.10.3 Thin Layer Chromatography:

22.10.3.1 General TLC solvent systems:
MeOH:NH_4OH (100:1.5)
$CHCl_3$:MeOH:HOAc (75:20:5)

22.10.3.2 Over-sprays:

22.10.3.2.1 Ninhydrin turns primary and secondary amines pink

22.10.3.2.2 Iodoplatinate

22.10.3.2.3 Potassium Permanganate ($KMnO_4$)

22.10.3.2.4 Marquis Reagent

22.10.4 FTIR

22.10.4.1 Extraction may be necessary to obtain a good spectrum for comparison.

22.10.4.2 See FTIR Test Method

22.10.5 GC/MS

22.10.5.1 Methamphetamine must have m/z 148 ion.
22.10.5.2 See GC/MS Test Method.

22.10.6 GC-IR

22.10.6.1 Extraction may be necessary to obtain good chromatography
22.10.6.2 See GC-IR Test Method

22.11 Report Writing:

22.11.1 Reference identification of marked Pseudoephedrine or Ephedrine tablets is sufficient, unless the charges are manufacturing.
22.11.2 See General Drug Identification Test Method**.**

22.12 References:

22.12.1 Indiana Criminal Code (scheduling)

22.12.2 Amphetamine CLIC Monographs

22.12.3 Validation of Pseudoephedrine/Ephedrine Quantitation Method, Early, K. (Indiana State Police, Evansville, IN). October 2004.

22.12.4 Isolation and Identification of Drugs, Clarke, E.G.C., The Pharmaceutical Press, London. 1969.

22.12.5 Clarke's Isolation and Identification of Drugs, 2nd Edition; Clarke, E. G. C. The Pharmaceutical Press, 1986.

22.12.6 Clarke's Analysis of Drugs and Poisons. 3rd Edition; Clarke, E. G. C. The Pharmaceutical Press, 2004

22.12.7 The Merck Index, 8th Edition; Merck and Company, Inc. 1968

22.12.8 Spot Tests in Organic Analysis, Fiegl, F. and Anger, V., Elsevier Publishing, New York. 1966.

22.12.9 Drug Unit Resource Manual
22.12.9.1 Separation and Identification of Amphetamine or Methamphetamine in combination with Ephedrine or

Caffeine, Stinson, Samuel and Berry, Michael; Microgram, Vol. VII, No. 4 (April, 1974) p. 51.

22.12.9.2 The Identification of Propylhexedrine, Dal Cason, Terry A. (Drug Enforcement Administration), Microgram, Vol. XV, No. 4 (April 1982).

22.12.9.3 Extractions of Methamphetamine from Vick's Inhalers, O'Neil, Quinn, Kern, and Finley (Commonwealth of Virginia), Microgram, Vol. XII, No. 7 (July 1979).

22.12.9.4 Separation and Identification of Methamphetamine in Phentermine, Methamphetamine, Ephedrine and Caffeine "Mini-Bennies", Anderson, Gundy and Lorch (Michigan Department of Public Health, Lansing, MI), Microgram, Vol. IX, No. 7 (July 1976).

22.12.9.5 Separation of Caffeine, Ephedrine and Phentermine, Stall, Walter (US Army Criminal Investigation Laboratory, Fort Gordon, GA). Microgram, Vol. X, No. 1 (January, 1977)

22.12.9.6 Screening Test for Amphetamine, Fleischer, David (NYC Police Department, New York, NY), Microgram, Vol. VIII, No. 8 (August, 1975).

22.12.9.7 Identification of Cathinone and Methcathinone, Dal Cason, Terry A., Microgram, Vol. XXV, No. 12, (December 1992).

22.12.9.8 Analysis of Phentermine/ Methamphetamine/ Ephedrine/ Caffeine Mixtures by GC/MS, Smith, R. Martin (Wisconsin Department of Justice, Madison, WI), Microgram, Vol. IX, No. 4, (April, 1976)

23. Phencyclidines and Ketamine:

23.1 Scope: Phencyclidine (PCP) and Ketamine are animal tranquilizers. These are frequently found in powder, crystal or liquid form. Both have been found in tablets and capsules and in Marijuana cigarettes. Several analogs of PCP exist and have been found in casework.

23.2 Precautions/Limitations:

23.2.1 PCP does not visualize well under UV for Thin Layer Chromatography.

23.2.2 PCP extract may need to be run on salt plates for FTIR.

23.2.3 Several phencyclidine analogs exist.

23.2.4 Multiple peaks may be present in GC/MS analysis. These peaks may be from precursors or breakdown products of the phencyclidines. The analyst should be aware of these substances and extend GC runs to allow for the parent compounds to elute from the GC column.

23.2.5 The same GC issues in 23.2.4 apply for GC-IR as well.

23.3 Related Information:

23.3.1 Appendix 1 – Forms and Worksheets
23.3.2 Appendix 2 – Abbreviations
23.3.3 Appendix 3 – Definitions
23.3.4 Appendix 4 – Drug Unit Reagent Preparation Manual
23.3.5 Other Test Methods
- **23.3.5.1** General Drug Identification
- **23.3.5.2** Color Tests
- **23.3.5.3** UV
- **23.3.5.4** TLC
- **23.3.5.5** Separations
- **23.3.5.6** FTIR
- **23.3.5.7** GC/MS
- **23.3.5.8** GC-IR

23.4 Instruments:

23.4.1 UV
23.4.2 FTIR
23.4.3 GC/MS

23.4.4 GC-IR

23.5 Reagents/Materials:

23.5.1 Color Test Reagents
23.5.2 TLC Solvent Systems
23.5.3 Methanol
23.5.4 $CHCl_3$

23.6 Hazards/Safety:

23.6.1 Inhalation/Exposure hazards – Ether (liquid form)
23.6.2 Chemical Hazard – Cyanide Precursor, Use of acids with PCP may potentially release cyanide gas.

23.7 Reference Materials/Controls/Calibration Checks:

23.7.1 Appropriate Reference Materials for Phencyclidine, Ketamine, or other drug of interest.

23.8 Procedures/Instructions:

23.8.1 Color Tests:
23.8.1.1 $Co(SCN)_2$ (See Reagent Preparation Guide)

23.8.2 Extractions:
23.8.2.1 PCP may be extracted with organic solvents from aqueous alkaline solutions.

23.8.2.2 PCP may be extracted from plant materials by washing the plant material with a suitable solvent (hexane, methanol, etc.) and filtered. It may be necessary to extract further to remove color from the sample.

23.8.2.3 Ketamine may be extracted with organic solvents from aqueous alkaline solutions.

23.8.2.4 May be dry-extracted with methanol or other organic solvents.

23.8.3 TLC Systems: General Acid and Base systems are sufficient:
$MeOH:NH_4OH$ (100:1.5)
$CHCl_3$:MeOH:HOAc (75:20:5)

Oversprays: Ninhydrin (if desired), iodoplatinate.

23.8.4 FTIR, if possible. Extraction may be necessary.

23.8.5 GC/MS – PCP analogs can be separated at appropriate temperatures. Ketamine generally chromatographs well. A general temperature program may be appropriate. See GC/MS Test Method.

23.8.6 GC-IR – General temperature programs may be appropriate. See GC-IR Test Method.

23.9 Records: See General Drug Identification Test Method.

23.10 Interpretations of Results:

23.10.1 Color Tests

23.10.1.1 $Co(SCN)_2$ turns blue with Phencyclidine

23.10.1.2 (Morris Test) Basified $Co(SCN)_2$ turns lavender with Ketamine HCl.

23.10.2 UV (in acid) – strong UV absorbers.

23.10.2.1 PCP near triplet with maximum at 262nm with extra shoulder at approximately 250nm.

23.10.2.2 Ketamine near triplet with maximum at 269nm, 276nm with shoulder at approximately 260nm.

23.10.3 TLC: Ninhydrin over-spray is good for detecting PCP and Ketamine.

23.10.4 FTIR – See FTIR Test Method

23.10.5 GC/MS – See GC/MS Test Method and 23.2.4 for precautions.

23.10.6 GC-IR – See GC-IR Test Method and 23.2.5 for precautions.

23.11 Report Writing: See General Drug Identification.

23.12 References:

23.12.1 Indiana Criminal Code

23.12.2 PCP: The Threat Remains, DEA Intelligence Division, Microgram, Vol. XXXVI, No.8, August 2003.

23.12.3 The Identification of N-ethyl-1-phenylcyclohexylamine Hydrochloride (Cyclohexamine), Barron, R.P. (DEA – Special Testing Laboratory), Sept.1973

23.12.4 1-Pyrrolodinocyclohexane Carbonitrile and Intermediate to the Pyrrolidine Analog of Phencyclidine, Teets, Barbara S. (Virginia Department of General Services, Bureau of Forensic Sciences, Merrifield, Virginia), Microgram, Vol. XIX, No. 8., August 1986

23.12.5 1-Piperidinocyclohexane Carbonitrile A Phencyclidine Precursor, Siefert, John. H. (Michigan State Police Crime Detection Laboratory, Madison Heights, MI), Microgram Vol. X, No. 7, July 1977.

23.12.6 Thiophene Analog of Phencyclidine, Alvarez, Jose (DEA Laboratory Notes), Microgram, Vol. X, No. 9, September 1977.

23.12.7 Thiophene Analog of PCP, Heagy, James (San Francisco Regional Laboratory, San Francisco, CA), November 1972.

23.12.8 Differentiation of PCP, TCP, and a Contaminating Precursor PCC, by Thin Layer Chromatography, Shulgin, Alexander.

23.12.9 Analysis and Identification of Phencyclidine Hydrochloride (PCP, Sernyl), DeZan, Paul and Bianchi, Robert (US Food and Drug Administration, New York)

23.12.10 PCP Purification, Huttsell , Fred L. (Indiana State Police Laboratory, Indianapolis, IN)

23.12.11 A Spectroscopic and Chromatographic Study of Phencyclidine (PCP) and Its Analogs, Rao, Soni, and Mullen (Baltimore Police Department, Baltimore, MD), Microgram, Vol. XIII, No. 4 , April 1980.

23.12.12 Purification and Identification of Phencyclidine, Johns, Susan Hart and Bubonic, John (Illinois Bureau of Identification, Perkin, IL), Microgram, Vol. X, No.7, July 1977.

23.12.13 Analysis and Identification of 1-[1-(2-thienyl) cyclohexyl]piperidine (TCP), Picard, David R. (Wisconsin Crime Laboratory Bureau, Madison, WI)

23.12.14 The Identification of a New Analog of PCP, 1-(1-phenylcyclohexyl)pyrrolidine (PCPy), Morris, Wayne (Florida Department of Criminal Law Enforcement, Jacksonville, FL), Microgram, Vol. X, No. 11, November 1977.

24. Clandestine Laboratory Sample Examinations:

24.1 Scope: Samples from clandestine laboratory reaction mixtures require unique analysis and sampling procedures. Knowledge of procedures being utilized is important. Examination and identification of precursor compounds and finished product are necessary, as well as identification of intermediate products in some cases. Analysis and subsequent identification of inorganic compounds, including acids and bases, may require the transfer of certain items to the Microanalysis Unit.

The submitted items of evidence should collectively contain the necessary components to fully demonstrate either the intent to manufacture or the successful manufacture of a controlled substance. In addition to the controlled substance which is suspected to be the primary product, precursors should be identified when present.

24.2 Precautions/Limitations: Items of evidence submitted from clandestine labs are often liquids containing volatile, flammable, and toxic chemicals as well as suspected drugs, volatile samples, complex media, intermediates, small amounts of materials, hazardous chemicals, and potential reactions.

24.3 Related Information:

- **24.3.1** Appendix 1 –Forms and Worksheets
- **24.3.2** Appendix 2 – Abbreviations
- **24.3.3** Appendix 3 – Definitions
- **24.3.4** Appendix 4 – Drug Unit Reagent Preparation Manual
- **24.3.5** Other Test Methods
  - **24.3.5.1** Phenethylamines
  - **24.3.5.2** Methoxyamphetamines
  - **24.3.5.3** Phencyclidines and Ketamine
  - **24.3.5.4** General Drug Identification
  - **24.3.5.5** Separations and Extractions
  - **24.3.5.6** Evidence Handling
  - **24.3.5.7** Sampling
  - **24.3.5.8** UV
  - **24.3.5.9** FTIR
  - **24.3.5.10** GC/MS
  - **24.3.5.11** GC-IR

24.4 Instruments:

- **24.4.1** UV
- **24.4.2** FTIR
- **24.4.3** GC/MS
- **24.4.4** GC-IR

24.5 Reagents/Materials:

24.5.1 Color Test Reagents
24.5.2 TLC Solvent Systems
24.5.3 Water finding paper
24.5.4 pH paper
24.5.5 Methanol
24.5.6 $CHCl_3$

24.6 Hazards/Safety: This type of evidence can pose significant health hazards that are not commonly encountered with routine controlled substance examinations. These hazards may include but are not limited to: corrosives, caustic materials, explosives, toxic gases, and flammable solvents.

Caution should be exercised when opening and examining evidence of this nature by utilizing appropriate personal protective equipment and sampling in a fume hood. Every effort should be made to prevent exposure to potentially hazardous materials. Special storage precautions may be necessary.

See General Drug Identification Test Method

24.7 Reference Materials/Controls/Calibration Checks:

24.7.1 Appropriate Reference Materials for drugs of interest.

24.8 Procedures/Instructions:

24.8.1 These items of evidence can consist of multiple layers of liquid. Determine if the liquids are aqueous or organic in nature. Check the pH of the aqueous layer prior to proceeding.

When the aqueous layer is acidic, then basic drugs will be in the aqueous layer and not in the organic layer. If the aqueous layer is basic, then basic drugs will be in the organic layer. The liquid layer suspected of containing the drug of interest will be examined, and the two layer liquids will not routinely be examined as separate sub-items.

24.8.2 After sampling organic liquids from clandestine lab evidence, the analysts should fume organic liquids with hydrochloric acid to convert free base amines (amphetamine and methamphetamine) to the stable hydrochloride salt. Organic liquids should then be evaporated in a fume hood prior to any examinations. This process is to minimize the hazards to laboratory personnel from ether and other solvents from clandestine labs.

24.8.3 After evaporation of organic liquids, the examination of the resulting residue could use the same procedures as "general unknown" solid or residue drug evidence.

24.8.4 Due to the nature of clandestine labs and the need to identify precursors, analysts may be required to confirm the identity of Ephedrine or Pseudoephedrine in clan lab samples. It is sufficient to identify items containing either Ephedrine or Pseudoephedrine, without the requirement of purification for specific drug identification by infrared spectroscopy. If methamphetamine and ephedrine/pseudoephedrine are present in a mixture, it is not necessary to confirm or indicate the ephedrine/pseudoephedrine.

24.9 Records: See General Drug Identification.

24.9.1 Results of pH and water finding paper testing, as well as the dates of these tests, shall be documented in the analytical notes.

24.9.2 Extraction and sample preparation procedures used shall be documented in the analytical notes.

24.10 Interpretations of Results: See General Drug Identification Test Method

24.11 Report Writing: See General Drug Identification Test Method

24.11.1 Results in the report can be stated as "found to contain Ephedrine and/or Pseudoephedrine" if the specific drug has not been identified.

24.12 References:

24.12.1 Drug Unit Resource Manual – Phenethylamines

24.12.1.1 Impurities In Methamphetamine Manufactured From Over-The-Counter Pseudoephedrine Tablet Preparation, Melgoza, Lynn (California Department of Justice, Riverside, CA) Journal of the Clandestine Laboratory Investigating Chemists Association, Vol. 9, No. 2-3, April-July, 1999.

24.12.1.2 A Field Test for Phenyl-2-Propanone, Kiser, Wilmer (DEA, Southeast Laboratory), Microgram Vol. XV, No. 9 (August, 1982).

24.12.1.3 Some Information Regarding Phenyl-2-Propanone, Dal Cason, Terry A. (DEA Central Laboratory, Chicago, IL), Journal of The Clandestine Laboratory Investigating Chemists Association (CLIC), Vol. 4, No. 1, January 1994.

24.12.2 Isolation and Identification of Drugs, Clarke, E.G.C., The Pharmaceutical Press, London. 1969.

24.12.3 Clarke's Isolation and Identification of Drugs, 2nd Edition; Clarke, E. G. C. The Pharmaceutical Press, 1986.

24.12.4 Clarke's Analysis of Drugs and Poisons. 3rd Edition; Clarke, E. G. C. The Pharmaceutical Press, 2004

24.12.5 Basic Training Program for Forensic Drug Chemists, Canaff, BNDD

24.12.6 Analytical Profiles of Amphetamines and Related Phenethylamines, CND Analytical

24.12.7 Forensic Investigation of Clandestine Laboratories, Donnell R. Christian, CRC Press

24.12.8 CLIC Journal (past issues)

24.12.9 Clandestine Laboratory Resource Articles

24.12.10 Indiana State Police Clandestine Laboratory Training Program

25. Opiates:

25.1 Scope: Members of this drug class are naturally occurring alkaloids of the Papaver somniferum poppy, and their semi-synthetic derivatives. These include, but are not limited to, Morphine, Heroin, Codeine, Opium, Hydrocodone, Oxycodone, Dextropropoxyphene, Dextromethorphan and Methadone.

25.2 Precautions/Limitations:

25.2.1 While most members of this group are easily extracted using organic solvents from aqueous alkaline solutions, Morphine extraction is pH sensitive and requires a weakly basic solution.

25.2.2 It is necessary to determine the optical isomer of Propoxyphene and Methorphan, when practicable, since one isomer of these substances is not controlled. (See Polarimetry and/or Melting Point Determination Test Methods)

25.2.3 The UV absorbances of some members of this group resemble Phenethylamines more than the rest of the opiates.

25.2.4 Certain members of this group appear in more than one controlled substance schedule. The presence of other substances may dictate the scheduling of the preparation.

25.2.5 Opium is a naturally occurring material that contains a variety of alkaloids. A Meconic acid color test is required for Opium identification to support its natural origin.

25.2.6 Special considerations may be needed when the sample contains Heroin and aggregate weight thresholds apply. For GC/MS, in the TIC, if the Heroin peak is less than 10% of the peak with the highest abundance, no further attempt to confirm Heroin needs to be made. If it is greater than 10%, at least one additional attempt should be made to confirm Heroin, depending on the complexity of the mixture.

25.3 Related Information:

25.3.1 Appendix 1 – Forms and Worksheets
25.3.2 Appendix 2 – Abbreviations
25.3.3 Appendix 3 – Definitions
25.3.4 Appendix 4 – Drug Unit Reagent Preparation Manual

25.3.5 Other Test Methods
- **25.3.5.1** General Drug Identification
- **25.3.5.2** Color Tests
- **25.3.5.3** UV
- **25.3.5.4** TLC
- **25.3.5.5** Separations
- **25.3.5.6** FTIR
- **25.3.5.7** GC/MS
- **25.3.5.8** GC-IR
- **25.3.5.9** Polarimetry
- **25.3.5.10** Melting Point Determination

25.4 Instruments:
- **25.4.1** UV
- **25.4.2** FTIR
- **25.4.3** GC/MS
- **25.4.4** GC-IR
- **25.4.5** Polarimeter
- **25.4.6** Melting Point Apparatus

25.5 Reagents/Materials:

- **25.5.1** Color Test Reagents
- **25.5.2** See Other Test Methods

25.6 Hazards/Safety: See individual drug and chemical MSDS**.**

25.7 Reference Materials/Controls/Calibration Checks:

- **25.7.1** Appropriate Reference materials for drugs of interest.

25.8 Procedures/Instructions:

- **25.8.1** Extraction: Most opiates can be extracted from aqueous alkaline solutions with organic solvents.
  - **25.8.1.1** Morphine and some derivatives of Morphine are an exception. It is pH sensitive and its sulfate form is not soluble in $CHCl_3$. Morphine can also be run direct in MeOH. See Separation and Extractions Test Method and Drug Unit Resource Manual(s).

25.8.1.2 Heroin can be extracted with $CHCl_3$ from 1 N HCl or from aqueous alkaline solutions.

25.8.2 Color Tests

25.8.2.1 Opium color test (Meconic acid test).

25.8.3 Optical Isomer Determination: The optical isomer of Propoxyphene shall be determined. The optical isomer of Methorphan, or other opiate, should be determined, if necessary. (See Polarimetry and/or Melting Point Determination Test Methods).

25.9 Records: See Other Test Methods.

25.10 Interpretations of Results:

25.10.1 Color Tests:
25.10.1.1 Marquis turns purple for opiates
25.10.1.2 Mecke's yellow green- turquoise for most opiates

25.10.1.3 Meconic Acid test: turns red with opium

25.10.2 UV: generally around 280 nm +/- 2nm;
Propoxyphene resembles the UV spectra of phenethylamines

25.10.3 TLC: General Acid and base systems
MeOH: NH4OH (100:1.5)
$CHCl_3$: MeOH: HOAc (75:20:5)

Over-sprays: Ninhydrin, Iodoplatinate, Potassium Permanganate

25.10.4 FTIR: See FTIR Test Method

25.10.5 GC/MS: See GC/MS Test Method

25.10.6 GC-IR: See GC-IR Test Method

25.10.7 Polarimetry: See Polarimetry Test Method

25.10.8 Melting Point Determination: See Melting Point Determination Test Method

25.11 Report Writing:

25.11.1 Samples may be reported as Opium if the Meconic acid test is positive, Morphine has been identified, and at least one other Opium alkaloid in the sample has been identified. Other Opium alkaloids are not required to be identified, but should be indicated.

25.11.2 The presence of non-controlled substances in a pharmaceutical preparation that contains a controlled substance may change the schedule of the controlled substance. Examples are Dextropropoxyphene and Acetaminophen, and Codeine with Aspirin or Acetaminophen. In this situation, both drugs shall be listed in the report. Non-controlled substances can be reported as indications and are not required to be confirmed. (See 4.10.7 and 4.10.8).

25.11.3 Optical Isomer Determination: See Polarimetry and/or Melting Point Determination Test Methods.

25.11.4 All others, See General Drug Identification Test Method

25.12 References:

25.12.1 Drug Unit Resource Manual(s)

25.12.1.1 Identification of Dextropropoxyphene and its Diastereomers, Newby, N.R and Hughes, Journal of Forensic Sciences, JFSCA, Vol. 25, No. 3, July, 1980, pp.646-654.

25.12.1.2 Extraction of Dextropropoxyphene from Pharmaceutical Mixtures, Gundy, E., Kemppainen, A. (Michigan State Police), Microgram, Vol. XII, No.6, June 1979.

25.12.1.3 Purification and Identification of Clandestinely Synthesized Mecloqualone, Dal Cason, T., Microgram, Vol. IX, No. 12, December, 1976.

25.12.1.4 GC-MS Identification of Methaqualone, Nowicki, H., Microgram, Vol. IX, No. 9, September, 1976.

25.12.1.5 A Color Test for the Detection of Methaqualone, Medina, F. and Goldson, B., Microgram, Vol. XIV, No. 4, April, 1981.

25.12.2 Determination of Codeine in Cough Syrups, Van Sickle, Department of Justice Drug Enforcement Administration, Chicago, IL

25.12.3 Chromatographic and Electrochemical Investigations of Codeine, Meinsma and Kissinger, Purdue University, 1985.

25.12.4 Quantitation of Codeine in Cough Syrup, Netsch, S. (Indiana State Police), January 1986.

25.12.5 The Synthetic Drug 3-methylfentanyl: Identification and Quantitation of Powdered Samples, Esposito and Winek, Journal of Forensic Sciences, Vol 36, No 1, Jan 1991, p86-92

25.12.6 BNDD Analytical Manual: Analysis of Drugs (initial issuance), United States Department of Justice Bureau of Narcotics and Dangerous Drugs.

26. Barbiturates and Hypnotics:

26.1 Scope: Barbiturates are substituted derivatives of Barbituric acid. Examples include, but are not limited to Barbital, Butalbital, Pentobarbital, Secobarbital, Amobarbital, Butabarbital and Phenobarbital. These substances are most commonly found in pharmaceutical preparations. Hypnotics generally include a variety of substances such as Methaqualone, Gamma Hydroxybutyric Acid (GHB), Choral Hydrate and Ethchlorvynol.

26.2 Precautions and Limitations:

26.2.1 Reproducibility of infrared spectral data may be difficult due to the presence of multiple crystalline forms (polymorphism).

26.2.2 Barbiturates generally do not absorb during UV analysis when run in aqueous acid solutions. UV analysis of barbiturates should be run in aqueous alkaline solutions.

26.2.3 GHB and its lactone, Gamma Hydroxy butyrolactone (GBL) exist in equilibrium with each other. It is very easy to convert one to the other depending on the pH of the sample or application of heat.

26.2.4 GHB is very hygroscopic and may need to be carefully dried prior to IR analysis.

26.2.5 Some drugs may be suspended in oils, or other viscous liquid. Extraction is necessary for analysis.

26.2.6 It may be necessary to extract the sample before proceeding with instrumental analysis.

26.3 Related Information:

26.3.1 Appendix 1 – Forms and Worksheets
26.3.2 Appendix 2 – Abbreviations
26.3.3 Appendix 3 – Definitions
26.3.4 Appendix 4 – Drug Unit Reagent Preparation Manual
26.3.5 Other Test Methods
- **26.3.5.1** Color Tests
- **26.3.5.2** UV
- **26.3.5.3** TLC

26.3.5.4 FTIR
26.3.5.5 GC/MS
26.3.5.6 GC-IR
26.3.5.7 General Drug Identification
26.3.5.8 Separation and Extractions

26.4 Instruments:

26.4.1 UV
26.4.2 FTIR
26.4.3 GC/MS
26.4.4 GC-IR

26.5 Reagents/Materials:

26.5.1 Color Test Reagents - See Color Test Method
26.5.2 TLC Systems – See TLC Test Methods

26.6 Hazards/Safety: See MSDS for individual drugs and chemicals.

26.7 Reference Materials/Controls/Calibration Checks:

26.7.1 Appropriate reference materials for drug(s) of interest.

26.8 Procedures/Instructions: See General Drug Identification

26.8.1 Extraction: Barbiturates are extracted from either acidic or weak basic aqueous solutions with organic solvents.

26.8.2 Complex mixtures suspected to contain GHB may need to be derivatized to preserve the form of the substance.

26.8.3 GHB Derivatization Procedure:

26.8.3.1 Sample extraction: Add HCl to pH ~2;
26.8.3.2 Add NaCl to form GHB sodium salt if GHB present.
26.8.3.3 Extract with Ethylacetate and evaporate to dryness.
26.8.3.4 Reconstitute dried extract with CH_2Cl_2 (do not use MeOH)
26.8.3.5 Add 10 drops 99%BSTFA w/1%TMCS to 10 drops of extracted sample in CH_2Cl_2.
26.8.3.6 Cork and parafilm test tube, heat to 60°C for 10 minutes.
26.8.3.7 Inject onto GC/MS (or GC-IR) using a 90-280°C program.

26.8.4 Exempt Preparations: See General Drug Identification 4.10.10.

26.8.5 pH of GHB should be neutral, approximately pH 6-7

26.8.6 Color Tests
26.8.6.1 Barbiturates: Dille-Koppanyi
26.8.6.2 GHB : 5% Ferric Chloride or 1% Cobalt Nitrate

26.8.7 UV
26.8.7.1 Barbiturates: 0.45 N NaOH.
26.8.7.2 GHB: MeOH
26.8.7.3 Methaqualone: 0.5N H_2SO_4

26.8.8 TLC Systems
26.8.8.1 Barbiturates: $CHCl_3$:Acetone (9:1); Over-sprays: saturated mercurous nitrate, potassium permanganate ($KMnO_4$), Diphenylcarbazone

26.8.8.2 GHB : Water: MeOH (1:1); Over-spray with 5% Ferric Chloride

26.8.9 FTIR: Difficulties with polymorphism of barbiturates can be circumvented by subjecting the drug reference material and the unknown sample to the same extraction procedures.

The hygroscopic nature of GHB may make IR analysis difficult. Dry the sample with low heat to drive off residual water.

26.8.10 GC/MS: GHB converts to the lactone in the injection port. Derivatization with BSTFA or BSTFA-TMCS may be required to confirm the presence of GHB.

26.8.11 GC-IR: This technique has the same limitations as with GC/MS.

26.9 Records: See General Drug Identification.

26.10 Interpretations of Results:

26.10.1 Color Tests:
Dille-Koppanyi turns purple/violet in the presence of barbiturates.

The Ferric Chloride test turns rust-red in the presence of GHB. GBL and the butanediols do not react to this test.

26.10.2 UV

26.10.2.1 Barbiturates: (in base) 254nm
26.10.2.2 GHB: (in MeOH) 209nm
26.10.2.3 GBL: (in MeOH) 211nm

26.10.3 TLC Systems – See TLC Test Method/ Reagent Prep Manual

26.10.4 Over-sprays:

26.10.4.1 Barbiturates:

KMnO4 – reacts with barbiturates, give yellow spots on a purple background.

HgSO4 – spray heavily to give light spots on an off-white background

Diphenylcarbazone – overspray for mercuric sulfate, turns barbs pink

26.10.4.2 GHB – Over-spray with 5% Ferric Chloride

26.10.5 FTIR may require extraction to be performed prior to this type of analysis. Acceptable FTIR spectral comparisons may be difficult due to the polymorphism of barbiturate samples.

Excess water may affect GHB FTIR spectral comparisons and may necessitate drying the sample under low heat prior to performing this type of analysis. See FTIR Test Method.

26.10.6 Analysis by GC/MS and GC-IR may require extraction. Derivatization may be necessary for GHB samples. (See 27.8.3) See GC/MS and /or GC-IR Test Methods.

26.11 Report Writing: See General Drug Identification Reporting – 4.11

26.11.1 It may be necessary to use a combination of statements to accurately describe the analysis results for exempt preparations. (See 4.11.6 and 4.11.7).

26.12 References:

26.12.1 Analytical Profiles of Barbiturates and Other Depressants, CND Analytical, Inc., Auburn , AL 1991

26.12.2 Separation and Identification of the Components of a Common Barbituric Acid Preparation, Stall, Walter (US Army Laboratory – San Francisco, CA); Microgram, Vol. XI, No. 12, December, 1978.

26.12.3 A Scheme for the Separation of Sandoptal (Butalbital) from "Fiorinal", Krautman, K. and Nanneman, D. (Missouri State Highway Patrol); Microgram, Vol. XIII, No. 12, December, 1980.

26.12.4 Drug Unit Resource Manual(s)

26.12.5 Validation of GHB Color Test Method, Nickless, R. (Indiana State Police), 2004.

27. Benzodiazepines:

27.1 Scope: Benzodiazepines are usually found in tablet or capsule preparations that have been diverted from legitimate sources. Analysis of a marked dosage unit generally consists of a reference identification and subsequent confirmation of the active ingredient(s).

27.2 Precautions/Limitations:

27.2.1 Presumptive color (spot) tests do not react with benzodiazepines.

27.2.2 Multiple TLC systems are suggested due to the variety of benzodiazepines.

27.2.3 There is not a specific visualization reagent for benzodiazepines.

27.2.4 Clorazepate decarboxylizes to Desmethyldiazepam in the GC/MS. FTIR is the recommended method of confirmation, if possible.

27.2.5 Counterfeit tablets marked as containing benzodiazepines have been encountered in casework.

27.3 Related Information:

27.3.1 Appendix 1 – Forms and Worksheets
27.3.2 Appendix 2 – Abbreviations
27.3.3 Appendix 3 – Definitions
27.3.4 Appendix 4 – Drug Unit Reagent Preparation Manual
27.3.5 Other Test Methods
- **27.3.5.1** General Drug Identification
- **27.3.5.2** UV
- **27.3.5.3** TLC
- **27.3.5.4** FTIR
- **27.3.5.5** GC/MS
- **27.3.5.6** GC-IR
- **27.3.5.7** Separations

27.4 Instruments:

27.4.1 UV
27.4.2 FTIR
27.4.3 GC/MS
27.4.4 GC-IR

27.5 Reagents/Materials: See Other Test Methods

27.6 Hazards/Safety: See Other Test Methods and MSDS.

27.7 Reference Materials/Controls/Calibration Checks:

27.7.1 Appropriate reference materials for drug(s) of interest.

27.8 Procedures/Instructions: See General Drug Identification Test Method.

27.8.1 Extraction: Most benzodiazepines are soluble in Methanol. However some extractions work better using $CHCl_3$. Dry extractions with $CHCl_3$ generally work well. See Drug Unit Resource Manuals for other options.

27.8.2 Clorazepate: Extraction options will yield the monopotassium form of the drug or Desmethyldiazepam. FTIR is recommended for confirmation.

27.8.3 Recommended TLC Systems:

MeOH: NH_4OH (100:1.5)
$CHCl_3$: Acetone (80:20) or (9:1)
Cyclohexane: toluene: diethylamine (75:15:10)

Over-sprays: Iodoplatinate overspray

27.9 Records: See General Drug Identification Test Method.

27.10 Interpretations of Results:

27.10.1 General Benzodiazepines: See General Drug Identification Test Method.

27.10.2 Clorazepate

27.10.2.1 FTIR analysis can be used to confirm the presence of the monopotassium salt, rather than the original dipotassium salt.

27.10.2.2 GC/MS will give the spectrum of Desmethyldiazepam.

27.10.2.3 GC-IR will give a Desmethyldiazepam IR spectrum.

27.10.2.4 Markings can be used as an indicator of the drug contained in a capsule or tablet.

27.10.3 Ketazolam

27.10.3.1 Ketazolam degrades to Diazepam in the GC inlet.

27.10.3.2 Ketazolam and Diazepam can be distinguished using thin layer chromatography.

27.10.3.3 If a sample is not a legitimate pharmaceutical preparation and the data indicates the presence of Diazepam, additional testing shall be performed.

27.11 Report Writing: See General Drug Identification Test Method

27.11.1 If FTIR is the confirmatory technique for identification of a salt form of Clorazepate, it shall be reported as “found to contain Clorazepate, a controlled substance”.

27.11.2 If FTIR analysis is sufficient for either an indication or identification, and GC/MS is used for confirmation resulting in Desmethyldiazepam, the item may be reported as “Clorazepate, a controlled substance”.

27.11.3 If the item is a marked tablet or capsule, FTIR analysis is not possible or is of insufficient quality for identification, and GC/MS is used for confirmation resulting in Desmethyldiazepam, it may be reported as “Clorazepate, a controlled substance.”

OR

Either situation in 27.11.2 and 27.11.3 may also be reported as “Markings and examination were consistent with a preparation containing Clorazepate, a controlled substance.”

27.12 References:

27.12.1 Analytical Profiles of the Benzodiazepines, CND Analytical, Auburn, AL, 1989.

27.12.2 The Analysis of Controlled Substances, Cole, Michael D., Wiley, 2003.

27.12.3 Identification of Some Interferences in the Analysis of Clorazepate, Suzuki, E.M. and Gresham, W.R., JFS Vol 28., No. 3, July 1983, pp 655-682.

27.12.4 Isolation and Identification of Clorazepate, Suzuki, and Gresham (Washington State Patrol, Crime Laboratory Division, Seattle, WA), Microgram, Vol. XVII, No. 4, April 1984.

27.12.5 The Extraction and Analysis of Salts of Clorazepate, Siefert, John (Michigan Department of Public Health, Warren, MI), Microgram, Vol. X, No. 10, October 1977.

27.12.6 Extraction and Identification of Clorazepate Monopotassium from Clorazepate Dipotassium, Smith, George E. (Indiana State Police Laboratory, Indianapolis, IN)

27.12.7 Mass Spectra of Benzodiazepines, Huttsell, Fred L. (Memo from J. Forbes), July 1984.

28. Anabolic Steroids:

28.1 Scope: "Anabolic steroids" is the name given to a series of natural and synthetic substances whose primary effects are to promote skeletal muscle growth. Most of these substances also have varying "androgenic" effects; which increase male sexual characteristics. Anabolic steroids are controlled within Schedule III of the Federal Drug Code in the United States and are defined and listed as "any drug or hormonal substance chemically and pharmacologically related to testosterone (other than estrogens, progestins, and corticosteroids) that promotes muscle growth". The Indiana Controlled Substance schedule III also includes Anabolic Steroids (as defined in 21 U.S.C.802 (41)(A) and 21 U.S.C. 802(41)(B).

28.2 Precautions/Limitations:

28.2.1 Many are manufactured in foreign countries with minimal quality control and may not contain the substances listed as ingredients on the label.

28.2.2 Complex mixtures are common and present difficulties in separation.

28.2.3 Steroids for intramuscular injection are frequently found in oils, such as cottonseed, sesame, or soybean oils, and need to be extracted prior to analysis to avoid contaminating instrumentation.

28.2.4 Steroids have many synonyms and confusing nomenclature.

28.2.5 GC/MS analysis time may be lengthy due to the large size of the molecules. It is not uncommon to have a 30 minute GC run.

28.2.6 GC-IR analysis has the same limitations as GC/MS.

28.2.7 Oxymetholone reacts with Methanol and it is recommended to be run in $CHCl_3$ for the correct GC/MS data to be obtained.

28.3 Related Information:

28.3.1 Appendix 1 – Forms and Worksheets
28.3.2 Appendix 2 – Abbreviations
28.3.3 Appendix 3 – Definitions
28.3.4 Appendix 4 – Drug Unit Reagent Preparation Manual
28.3.5 Other Test Methods
- **28.3.5.1** General Drug Identification
- **28.3.5.2** Separation and Extractions
- **28.3.5.3** Color Tests

28.3.5.4 UV
28.3.5.5 TLC
28.3.5.6 FTIR
28.3.5.7 GC/MS
28.3.5.8 GC-IR

28.4 Instruments:

28.4.1 UV
28.4.2 FTIR
28.4.3 GC/MS
28.4.4 GC-IR

28.5 Reagents/Materials: See Other Test Methods

28.6 Hazards/Safety: See Other Test Methods

28.7 Reference Materials/Controls/Calibration Checks:

28.7.1 Appropriate reference materials for drug(s) of interest.

28.8 Procedures/Instructions:

28.8.1 Suggested Extraction:

28.8.1.1 Tablets – generally a filtered methanol extract will suffice.

28.8.1.2 Injectables – 1:1 ml mix with MeOH to the sample. Vortex. If top layer is not clear, cool in freezer for one hour and filter, while cold, into a clear beaker. Concentrate sample and perform testing procedures.

A methanol: distilled water (9:1) may work as well.

28.8.1.3 Immiscible Oils: add sodium bicarbonate/distilled H_2O to pH of approximately 8. Extract with $CHCl_3$, evaporate and run on GC/MS.

28.8.2 Thin Layer Chromatography

28.8.2.1 Chloroform: Ethyl Acetate (40:10), UV light box, with EtOH: H_2SO_4 (4:1) over spray

28.8.2.2 Chloroform: Acetone (9:1), UV light box, with Iodoplatinate, then $KMnO_4$; or EtOH: H_2SO_4 (4:1) over spray.

28.8.3 UV – See UV Test Method

28.8.4 FTIR – See FTIR Test Method

28.8.5 GC/MS – Long, high temperature programs, See GC/MS Test Method.

28.8.6 GC-IR – Long, high temperature programs would be necessary. See GC-IR Test Method.

28.9 Records: See General Drug Identification Method.

28.10 Interpretations of Results:

28.10.1 TLC: steroids show up well under short wave UV light.

28.10.2 UV spectrophotometry: typically around 240nm. Few have absorbances around 280nm and few will not give a significant UV spectrum.

28.10.3 FTIR – See FTIR Test Method

28.10.4 GC/MS – See GC/MS Test Method

28.10.5 GC-IR – See GC-IR Test Method

28.11 Report Writing: See General Drug Identification Test Method.

28.12 References:

28.12.1 21 Code of Federal Regulations - Chapter 11 - Food and Drugs, National Archives and Records Administration, Section 1308.02 Definitions, April 1, 1994.

28.12.2 Indiana Criminal Code 35-48-2-8(f).

28.12.3 United States Criminal Code, 21 USC 802(41)(A) and (41)(B).

28.12.4 Analysis of Anabolic Steroids, Koverman, Gary (Colorado Bureau of Investigation), Microgram, Vol. XXVI, No. 11, November 1993.

28.12.5 Screening of Steroids by Thin Layer Chromatography, Morley, M. and Matkovich, C. (DEA Mid-Atlantic Laboratory)

28.12.6 Analytical Profiles of the Anabolic Steroids and Related Substances (Vol. I), CND Analytical, Inc. Auburn, AL, 1989.

28.12.7 Analytical Profiles of the Anabolic Steroids and Related Substances (Vol. II), CND Analytical, Inc. Auburn, AL, 1991.

28.12.8 TLC Screen for Anabolic Steroids, FDA San Francisco Laboratory.

28.12.9 Black Market in Anabolic Steroids – Analysis of Illegally Distributed Products, Musshoff, Daldrup and Ritsch, Journal of Forensic Sciences 1997; 42(6):1119-1125.

29. Proficiency Testing:

29.1 Scope: Each forensic scientist conducting analysis in the Drug Unit shall participate in the Laboratory Division's proficiency testing program. Participation, evaluation, documentation, and any necessary corrective actions shall comply with procedures listed in the Laboratory Quality Assurance Manual. Procedures used for analysis of proficiency samples shall be similar to the procedures used for casework analysis and shall follow Drug Unit Test Methods. The following are guidelines for compliance with the proficiency testing program.

29.2 Precautions/Limitations: N/A

29.3 Related Information:

29.3.1 Appendix 1 – Forms and Worksheets
29.3.2 Appendix 2 – Abbreviations
29.3.3 Appendix 3 – Definitions
29.3.4 Appendix 4 – Drug Unit Reagent Preparation Manual
29.3.5 Other Test Methods

29.4 Instruments: N/A

29.5 Reagents/Materials:

29.5.1 External Proficiency Samples

29.5.2 Internal Proficiency Samples

29.6 Hazards/Safety: N/A

29.7 Reference Materials/Controls/Calibration Checks:

29.7.1 External Proficiency Testing: This sample shall be obtained from an approved test provider.

29.7.2 Open Internal Proficiency Testing: This sample can be either a proficiency sample prepared and distributed within the respective laboratory or an inter-laboratory proficiency sample.

All internal proficiency samples shall be prepared using known primary or secondary drug reference materials and may be mixed with various diluents and/or in various combinations of drugs to simulate street-type drug items.

29.7.3 Blind Proficiency Testing: If blind proficiency testing is to be conducted, the Drug Unit Supervisor(s) or the Section Supervisor shall prepare the blind proficiency samples using known primary or secondary drug reference materials mixed with various diluents and in various combinations of drugs to simulate street-type drug items.

29.7.4 Proficiency Re-analysis: Drug Unit Supervisors shall select drug case items for proficiency re-analysis from any forensic scientist's completed cases.

29.8 Procedures/Instructions:

29.8.1 External Proficiency Testing

Forensic Scientists in the Drug Unit shall participate in one open external proficiency test in drug analysis from an approved test provider annually. Exceptions to this procedure include trainees released for casework after all proficiencies have been distributed and analysts who are unavailable during the proficiency timeframe.

The Drug Unit Supervisors shall assign the annual external proficiency sample to a forensic scientist at each laboratory.

The forensic scientists are to complete the examination and forward the results, all notes and documentation to the Drug Unit Supervisor prior to the completion deadline.

29.8.2 Blind Internal Proficiency Testing

Each forensic scientist conducting drug analysis in the Drug Unit may be assigned drug items for blind proficiency testing.

Blind proficiency samples shall be submitted for analysis without the knowledge of the forensic scientists, evidence clerks, or Laboratory Managers at the respective laboratory. Blind proficiency samples shall be submitted as normal drug cases by police officers, and shall be assigned to forensic scientists for analysis by the Unit Supervisors.

The Drug Unit Supervisors shall prepare and distribute internal proficiency samples to analysts within their respective laboratories as deemed necessary.

29.8.3 Proficiency Re-analysis Samples

Drug case items previously examined by any forensic scientist may be selected for proficiency re-analysis.

Items selected can be assigned to any forensic scientist in the Drug Unit, except for the original examining forensic scientist.

All items for proficiency re-analysis shall be processed for analysis and chain of custody purposes as "regular" evidence. Chain of custody shall be documented via LIMS entries, and the forensic scientist shall comply with all procedures for marking and sealing of evidence. However, descriptions of evidence shall not be changed in the LIMS file. A comment shall be entered in the LIMS case file indicating that this item has been selected for proficiency re-analysis.

29.9 Records:

29.9.1 External and Internal Proficiencies: All notes, documentation, and results are to be returned to the Unit Supervisor by the assigned deadline. The Quality Assurance Coordinator is responsible to ensure the completed necessary documentation has been submitted to the external vendor for evaluation.

29.9.2 Blind Proficiency: Since Blind Proficiencies are treated as regular casework, records shall be maintained in the laboratory case file as if it were a normal case.

29.9.3 Proficiency Re-Analysis: After the re-analysis is complete, copies of all documentation including conclusions for drugs present in the item(s) from the re-analysis and the original analysis will be forwarded to the respective Drug Unit Supervisor. The re-analysis documentation will be stored in the original case file with the Certificate of Analysis and notes of the original analysis. A new Certificate of Analysis will not be created for the re-analysis.

29.9.4 A Proficiency Test Log shall be completed upon evaluation of the test results by the Unit Supervisor responsible for administering the proficiency. The appropriate forensic scientist shall be notified of the results as per the Laboratory Quality Assurance Manual.

29.10 Interpretations of Results:

29.10.1 External Proficiency: The Drug Unit Supervisor shall review and evaluate the analysis by comparing to the manufacturer's report when it becomes available.

29.10.2 Open Internal Proficiency: The Drug Unit Supervisor shall review and evaluate the analysis of the known material in the sample(s).

29.10.3 Blind Proficiency: The Drug Unit Supervisor shall review and evaluate the analysis of the known material in the sample(s).

29.10.4 Proficiency Re-Analysis: The Drug Unit Supervisor shall review and evaluate the re-analysis by comparing to the original analysis.

29.11 Report Writing: See 30.9.

29.12 References:

29.12.1 Lab QA Manual

29.12.2 SWGDRUG Guidelines

29.12.3 ABC Guidelines

30. Drug Reference Materials:

30.1 Scope: This Test Method is intended as a guide to the proper acquisition, verification, use, and storage of Drug Reference Materials (formerly known as Drug Standards) used for drug identification.

30.2 Precautions/Limitations:

30.2.1 Reference Materials may not be commercially available for comparison.

30.2.2 Analytical data may not be available for verification or authentication of identity.

30.3 Related Information:

30.3.1 Appendix 1 – Forms and Worksheets
30.3.2 Appendix 2 – Abbreviations
30.3.3 Appendix 3 – Definitions
30.3.4 Appendix 4 – Drug Unit Reagent Preparation Manual
30.3.5 Other Test Methods

30.4 Instruments:

30.4.1 UV
30.4.2 FTIR
30.4.3 GC/MS
30.4.4 GC-IR
30.4.5 Polarimetry
30.4.6 Melting Point

30.5 Reagents/Materials: See Other Test Methods.

30.6 Hazards/Safety: See MSDS / SDS for individual Reference Materials and chemicals.

30.7 Reference Materials/Controls/Calibration Checks:

30.7.1 Drug Reference Materials: All Drug Reference materials shall be identified by a source and lot number, and/or other assigned identifier.

30.7.2 Drug Reference Material Libraries: All reference collections of drug or other materials used for identification purposes, comparison and/or interpretation shall be documented, uniquely identified and controlled. This includes, but is not limited to, user generated libraries, purchased data libraries or libraries obtained from reputable sources.

30.7.3 All Drug Reference Materials shall be secured in a locked cabinet, refrigerator, or freezer accessible only to Drug Unit analysts and supervisors assigned to that Regional Laboratory. It is the responsibility of the Drug Unit personnel to maintain the security and integrity of the Drug Reference Materials.

30.7.3.1 If a refrigerator or freezer is not available in the Drug Unit, the Drug Reference Materials shall be stored in a Regional Laboratory's evidence storage refrigerator or freezer.

30.7.3.1.1 The analyst shall place the Drug Reference Materials in an appropriate container and seal it with initials on the seal(s), in such a way that it cannot be opened without obvious signs of tampering. The container shall be clearly labeled as Drug Reference Materials and that it is not evidence.

30.7.3.1.2 The analyst shall transfer the sealed and labeled container to an Evidence Clerk or Property Officer, who shall place it in an appropriate Regional Laboratory's refrigerator or freezer storage location.

30.7.3.1.3 When Drug Reference Materials are needed from Regional Laboratory's evidence storage refrigerator or freezer, the analysts shall request an Evidence Clerk or Property Officer to obtain the sealed container for them.

30.7.3.1.4 Only an analyst shall open the sealed containers with the Drug Reference Materials.

30.7.4 Drug Reference Material Transfers Between Laboratories:

30.7.4.1 The package shall be sealed.

30.7.4.2 The package shall be transferred from one lab to another via an ISP laboratory employee, or through a commercial delivery service with traceable shipping.

30.7.4.3 If the substance is controlled in Federal Schedules I or II, a DEA 222 form shall be completed for the transfer.

30.7.4.4 If the substance is controlled in Federal Schedules III, IV, V or by the State of Indiana, and/or is not a controlled

substance, the Indiana State Police Drug Transfer form shall be completed.

30.7.4.5 If the transfer involves non-Drug Unit personnel, then a Drug Reference Materials Record and Receipt form shall be completed and uploaded onto SharePoint. At a minimum, the initial form shall be uploaded to SharePoint by the originating lab and the completed form will be uploaded to SharePoint by the final receiving lab. The files shall be named using the following nomenclature: Drug Name(s)_Originating Lab_to_Destination Lab_Date. Example: LSD_FWRL_to_IRL_9-24-17

30.8 Procedures/Instructions:

30.8.1 All Drug Reference Materials shall be verified prior to use in casework.

30.8.1.1 It is not necessary to reverify a Reference Material if it has been verified at a different Regional Laboratory. A copy of the Reference Material verification paperwork shall be maintained at both labs.

30.8.2 Multiple containers of a Drug Reference Material that have the same lot number may share a Reference Material Testing Record regardless of when they were received by the laboratory. At least one container shall be verified prior to use in casework.

30.8.2.1 Each vial needs to be uniquely identified even if the vials are intended to be transferred to a different lab (A, B, C or 1, 2, 3).

30.8.2.2 Data generated to verify or re-verify the reference material needs to specify which vial was used (A, B, C or 1, 2, 3).

30.8.2.3 A Reference Material Testing Record does not need to be completed for each individual vial. The form is used to document the verification of the specific lot number.

30.8.2.4 Dates of receipt need to be documented on both the Reference Material Testing Record and the vials.

30.8.3 Expired Drug Reference Materials

30.8.3.1 An expired reference material can still be used if verified prior to use. The new expiration date shall be two years from the date of verification. The documented reverification date refers to the date the reference material was run.

30.8.3.2 Recommended retest dates that appear on the vendor's Certificate of Analysis shall be treated as expiration dates.

30.8.3.3 If it is discovered that an expired Reference Material has been used in casework, the analyst shall immediately notify the Unit Supervisor and reverify the Reference Material. The supervisor shall notify the Accreditation and Quality Assurance Manager.

30.8.4 A Drug Reference Material Testing Record shall be initiated for each Reference Material when it has been received by the laboratory.

30.8.4.1 An additional examiner shall review the initial verification data, and initial both the printout of the data and the Testing Record.

30.8.5 It is only necessary to verify a reference material one time on one instrument. It is not necessary to verify or document the verification of Reference Materials in the Reference Material Testing Record every time they are used in an analytical procedure.

30.8.6 Access to the Drug Reference Materials shall be restricted to the members of each respective laboratory Drug Unit.

30.8.7 All verifications or authentications shall be made by spectral comparison to a known literature reference in the following order:

30.8.7.1 Peer reviewed articles, libraries and data (SWGDRUG monographs, Forendex, etc.).
30.8.7.2 Manufacturers data
30.8.7.3 Data from other accredited laboratories with sources and lot numbers
30.8.7.4 Data from other ISP Regional Laboratories (requires supervisory approval).

Whenever possible, the literature source should have data from traceable materials and the source and lot number of the reference material being verified should be different from that of the literature source.

30.8.8 Comparisons of mass spectral or infrared data shall constitute the minimum requirements for verification of primary Reference Materials.

30.8.9 Secondary Reference Materials shall be authenticated by a relevant preliminary test and confirmed by either GC/MS or FTIR at a minimum.

30.8.10 Primary Reference Materials are preferred for case material identification when available. In the absence of a primary Reference

Material, secondary Reference Materials may be used for identification purposes.

30.8.11 Spectral data may be entered into the user generated libraries after verification or authentication.

30.8.12 When a Drug Reference Material has been consumed, the bottle shall be discarded after a notation has been made on the Reference Material Testing Record. The procedure shall be observed by an additional examiner. Both examiners shall initial the Reference Material Testing Record once the bottle has been discarded.

30.8.13 When a Drug Reference Material has been spilled, the following actions shall be taken:

1. Take a picture(s) of the spillage.
2. If possible, scoop the spilled reference material into a plastic bag and seal it (with initials). This is not required if the reference material is a liquid.
3. Take a picture of the plastic bag containing the spilled reference material.
4. Return the reference material and the sealed plastic bag to its designated storage area.
5. Send an email to the immediate supervisor and laboratory manager detailing what happened and include the picture(s) of the spill.
6. The supervisor will dispose of the plastic bag containing the spilled reference material in the same way as a consumed reference material (with documentation in the logbook and observed by another analyst)
7. The supervisor will write a memo about the spill. All of the documentation regarding the spill will be uploaded onto SharePoint and the hardcopy will be kept with the DEA paperwork.

30.9 Records:

30.9.1 Drug Reference Material Testing Record: Documentation of the receipt, identity, source and lot number, verification or authentication record(s) of each Drug Reference Material, the initials of the forensic scientist that performed the verification or authentication and the initials of the reviewer of the data. This record shall be available for each Drug Reference Material used for identification and maintained by each respective laboratory Drug Unit.

30.9.2 Instrumental data (original or copies) supporting verification and/or authentication of the Reference Material shall be attached to the Drug Reference Material Testing Record. It is not necessary to attach the blank for the reference material verification. The solvent used for verification needs to be documented on the data.

30.9.2.1 Re-verification or extension of expiration date information such as manufacturer re-certification date and/or analytical data shall be kept in the Drug Reference Material Testing Record.

30.9.2.2 Re-verification is only necessary when a Reference Material has expired, or is about to expire.

30.9.2.3 Reference Materials that do not have supporting verification data shall be reverified prior to use in casework. This data shall be attached to the Drug Reference Material Testing Record. It is not necessary to reverify the Reference Material if it is not being used in casework.

30.9.3 Manufacturer's analytical data (or copies) shall be attached to the Drug Reference Material Testing Record, if available.

30.9.4 Literature references or sources used for verification or authentication of Drug Reference materials shall be documented in the Drug Reference Testing Record. If applicable and reasonable, attach copies of the spectra to the Testing Record.

30.9.5 An evaluation by two examiners shall be performed before a Drug Reference Material is entered into the user generated library.

30.9.6 All entries in user libraries shall have the source and lot number of the Drug Reference Material included as part of the data file and printed on the spectrum.

30.9.7 Purchased spectral libraries may not have source and lot number information available. This is beyond the control of the ISP laboratory and these entries cannot be changed.

30.9.8 When a Drug Reference Material has been consumed and the bottle discarded, two analysts must be present and both shall initial and record the date the material was consumed and the container discarded on the Reference Material Testing Record.

30.9.9 The DEA 222 forms and internal transfer forms shall be filed in an accessible location and should be with or in close proximity to Federal Drug records, laboratory drug licenses, etc.

30.9.10 A Reference Material inventory shall be completed annually. The inventory shall include the name of the Reference Material, the manufacturer, the lot number, the location in the laboratory, and expiration date, if applicable. Completed inventories shall be uploaded to SharePoint.

30.9.11 Each laboratory shall designate a primary and secondary analyst who is in charge of the inventory and organization of Reference Materials. The analyst shall notify the Unit Supervisor who has been designated for this assignment.

30.10 Interpretations of Results:

30.10.1 A Drug Reference Material is acceptable for use in case material identification after it has been analyzed in the laboratory and its analytical data has been compared to literature and found to be satisfactory. It may be included in the user generated spectral libraries.

30.10.2 If the literature spectral comparison is unsatisfactory, the Drug Reference Material cannot be used for casework identification and the spectrum shall not be included in the user generated libraries.

30.11 Report Writing: N/A

30.12 References:

30.12.1 Methods of Analytical/Sampling Seized Drugs for Qualitative Analysis: Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations.

30.12.2 Indiana State Police Laboratory Quality Assurance Manual.

31. Drug Unit Case Reviews

31.1 Scope: This Test Method is intended as a guide for the proper review of Drug Unit case records (examination and administrative documentation).

31.2 General:

31.2.1 Technical Reviews**:** A technical review is an evaluation of examination records after the examination has been performed. This review may take place before or after the report has been released. This review consists of determining whether the appropriate examinations have been

performed, the conclusions are consistent with the recorded data and are within the scope of the discipline or category of testing.

31.2.2 Administrative Reviews: Administrative Reviews are reviews of administrative records and a cursory review of analytical information prior to the approval of the report. This is not intended to be a technical review, but may include Test Method compliance and technical matters.

31.3 Related Information:

31.3.1 Technical Review form
31.3.2 LIMS Module information
31.3.3 Drug Unit Case Review Training Manual

31.4 Procedures/Instructions:

31.4.1 The analyst is responsible for preparing accurate, complete and organized examination records (case notes). The analyst shall review documentation constituting the case file (examination and administrative records) for compliance with laboratory policy and procedures and technical accuracy prior to submitting for administrative or technical review.

31.4.2 When the analyst submits the case for administrative or technical review, it shall be considered completed. The Unit Supervisor shall be notified if additional analysis is performed after the case is submitted for administrative or technical review.

31.4.3 Administrative Reviews: At a minimum, this review shall include:

31.4.3.1 A review of the Certificate of Analysis including:

31.4.3.1.1 Spelling and grammatical accuracy;
31.4.3.1.2 A review of the Certificate of Analysis to ensure that all key information is included.
31.4.3.1.3 Results are properly reported as per Drug Unit Test Methods

31.4.3.2 A review of all administrative and examination records associated with the Certificate of Analysis to ensure that the records are uniquely identified.

31.4.3.2.1 Case number
31.4.3.2.2 Item number(s)

31.4.3.2.3 Analysts' initials or signature (does not apply to administrative documents)

31.4.3.3 At the completion of a case, an administrative review shall be conducted on all Certificates of Analysis prior to release to the contributing agency and/or officer.

31.4.4 Technical Reviews: At a minimum, all examination documentation shall be reviewed for the following:

31.4.4.1 All of the requirements for an Administrative review, AND
31.4.4.2 Records of Weight and Balance checks,
31.4.4.3 Weight measurements,
31.4.4.4 Uncertainty of Measurement,
31.4.4.5 Testing procedures used
31.4.4.6 Quality of data,
31.4.4.7 Test Method compliance,
31.4.4.8 Compliance with applicable Laboratory policies,
31.4.4.9 Use of appropriate reference materials, blanks and controls

31.4.5 Additional items that may be reviewed:

31.4.5.1.1 Evidence Seals and markings
31.4.5.1.2 Evidence receipts
31.4.5.1.3 Chain of Custody records

31.4.6 Technical reviews shall be conducted by individuals that have expertise gained through training and experience in the category of testing being reviewed and shall have knowledge of the Drug Unit's test methods.

31.4.7 Technical and Administrative reviews shall not be conducted by the author of the Certificate of Analysis.

31.4.8 Technical reviews shall be performed on new analyst cases as per the Drug Unit Training manual.

31.4.9 The Unit Supervisor, or authorized designee, shall conduct a technical case review on a minimum of 5 cases from each analyst on a quarterly basis.

31.4.9.1 If the analyst completes less than 5 cases in a quarter, all cases shall be reviewed.

31.4.9.2 Cases worked by supervisory staff shall be reviewed by another supervisor or analyst with expertise gained through training and experience in the discipline being reviewed.

31.5 Records:

31.5.1 Administrative Reviews: The individual completing administrative reviews shall mark the case as Administratively Reviewed in the LIMS module to affix their Permanent Employee (PE) number to the final report. When an individual is being trained to perform administrative reviews, a Drug Unit Administrative Review Form shall be completed for each case that was reviewed during the training process.

31.5.2 Technical Reviews: The individual completing the review shall complete a Drug Unit Technical Case Review form, noting any problem(s) found, and corrections or action(s) taken.

31.5.2.1 If the technical review takes place before the report is administratively reviewed, the individual completing the technical review shall mark the case as Technically Reviewed in the LIMS module.

31.5.2.2 If the technical review is performed after the report has been administratively reviewed and released, the reviewer shall fill in the Case Review tracking boxes in the “additional data” tab in the LIMS module.

31.5.3 An electronic copy of the form used for the Technical Review shall be appropriately named and saved in the respective case record in the LIMS system by the reviewer.

31.6 Interpretations of Results:

31.6.1 Changes to notes**:** If the correction requires change(s) to the case record, the analyst shall make the corrections in the electronic version of the examination record and upload the corrected electronic version of the examination record to LIMS using the appropriate nomenclature. All pages of the original electronic examination record shall be retained. If possible, the corrected file should be sent back to the original reviewer.

31.6.2 Changes to Reports:

31.6.2.1 Prior to release: If the correction requires a change to the report prior to the approval and release of the original report, the analyst shall make the correction, and send the corrected file back to the original reviewer, if possible.

31.6.2.2 After release: If the correction requires a change(s) to the Certificate of Analysis after the original report has been released, the analyst shall make the necessary correction(s) and issue an Amended Report. The case reviewer shall perform an Administrative Review on the Amended Report and shall ensure all necessary corrections were made**.**

31.6.3 Resolution of Conflicts of Opinion: (See Laboratory QA Manual)

31.6.3.1 Once a formal case review has begun, the same reviewer, if available, shall complete the process.

31.6.3.2 If the analyst and reviewer disagree, the analyst shall not seek a second reviewer. Both analyst and reviewer shall consult the Unit Supervisor to attempt to resolve the disagreement.

31.6.3.3 The Unit Supervisor shall be notified of substantive variations of opinions. The analyst, the reviewer, and Unit Supervisor shall discuss the examination results, interpretations/opinions and conclusions.

31.6.3.4 If the difference of opinion cannot be resolved, Unit Supervisor(s) shall notify the Section Supervisor and Quality Assurance Manager to pursue resolution as per Laboratory Quality Assurance Procedures.

31.6.3.4.1 After consultation with the Quality Assurance Manager, Section Supervisor, Unit Supervisor and other staff as necessary, the situation will be evaluated and a determination will be made if a corrective action is to be required.

APPENDIX 1 FORMS AND WORKSHEETS

The following are the templates for the Drug Unit worksheets and log sheets as found on SharePoint. Analysts shall use an Examination Worksheet when documenting their analysis. However, the analyst may customize these worksheets with the specifics for their analysis and respective laboratories. Additional sheets are acceptable when appropriately marked as per laboratory policies.

1. Balance Verification Log
2. Calibration Verification Log
3. Drug Reference Materials Record and Receipt Form
4. Drug Unit Administrative Review Form
5. Drug Unit Technical Review Form

6. Examination Worksheet – General
7. Examination Worksheet – Plant Material
8. Examination Worksheet – Plant Material (Indianapolis)
9. Examination Worksheet – Tablets
10. GC-IRD Calibration Verification Log
11. GC-IRD Multiple Run Worksheet
12. GC/MS Calibration Verification Log
13. GC/MS Multiple Run Worksheet
14. GC/MS Preventative Maintenance Log
15. IRL Polarimeter Verifiaction Log
16. Indiana State Police Drug Transfer Form
17. Personal Review Form
18. Reagent Preparation and Verification Log
19. Reference Material Testing Record
20. Request for Laboratory Examination Form

APPENDIX 2 ABBREVIATIONS

AC or Ac-Cod	Acetylcodeine	MJ or mj	Marijuana
acet	Acetaminophen	Meth	Methamphetamine
APAP	Acetaminophen	Methamp	Methamphetamine
amph	Amphetamine	neg	negative
ANOR	Alternate Non-aqueous Organic Ratio	NCS	no controlled substance
~ or approx	approximate	NCR or NR	no color reaction or no reaction
asv	autosampler vial	N/E	not examined
aq.	Aqueous	nwt	net weight
bkg	Background	OC	Oxycodone
cig	Cigarette	pharm.	pharmaceutical(s)
cap(s)	capsule(s)	pb	plastic bag
coc	Cocaine	pm	plant material / plant-like material
Conc, con	Concentrated	PE or Pet E	petroleum ether
CMP	1-(1',4'-cyclohexadienyl)-2-methyl Aminopropane	Pet Ether	petroleum ether
CS or cs	controlled substance	+ or pos	positive
CT or ct	Color test(s)	precip, ppt	precipitate
ć, cont or :	Containing	prog	program
Duq. Lev or D-L	Duquenois-Levine	ψ-eph	pseudoephedrine
DIB	Drug Identification Bible	pse, pseudo	pseudoephedrine
diss.	Dissolved	PTHIT	Phenyltetrahydroimidazothiazole, Levamisole, Tetramisole
DMS	Dimethylsulfone	pwd	powder
DPH	Diphenhydramine		
DOS	Date of Seizure	rec'd	received
eff, ◊	effervescence, gassing	ref	reference
eph or ephed	Ephedrine	rej	rejected
env	Envelope	ret	retention
equiv	Equivalent	Rm, ref mat	Reference material
		RT	retention time
evap	Evaporated	rxn	reaction
evid	Evidence	sat, or sat'd	saturated
ext or extr	Extraction	sch	schedule
gwt	gross weight	sp	sealed plastic
hrc	hand rolled cigarette	spb	sealed plastic bag
HC	Hydrocodone	sl	Slight
HER	Heroin	soln, or sol'n	Solution
HM	Hydromorphone	sub, subs	Substance

ind	Indicates	tgw	total gross weight
ingred.	Ingredients	tab(s)	tablet(s)
init.	initials/initialed	tnw	total net weight
inj.	Injection	v	Very
liq.	Liquid	V/E	visually examined
man.	manual	veg	Vegetation
MAM	monoacetylmorphine	ẃ	With
mat or mat'l	material	ziplock plastic bag	zpb
mfr	manufacturer		

APPENDIX 3 DEFINITIONS

1. Atomic Mass Unit (amu) - A unit of mass equal to 1/12 the mass of the most abundant isotope of carbon, carbon 12 (carbon which is assigned a mass of 12).

2. BSTFA - N,O-Bis(trimethylsilyl)trifluoroacetamide; a derivatization compound. It is the preferred reagent for trimethylsilylation of alcohols, alkaloids, amines and biogenic amines, carboxylic acids, phenols, and steroids.

3. BSTFA-TMCS - N,O-Bis(trimethylsilyl)trifluoroacetamide with Trimethylchlorosilane; a derivatization compound; used for amides, secondary amines, especially good for analyzing drugs of abuse – THC, morphine, PCP, etc. is the preferred reagent for trimethylsilylation of alcohols, alkaloids, amines, biogenic amines, carboxylic acids, phenols, and steroids.

4. Calibration - Adjusting a piece of equipment to a certain set of performance standards.

5. Chromophores - the molecular grouping that is responsible for UV absorption, usually a conjugated system (double bonds) where the electron density is spread out over the molecule.

6. Confidence level - the extent or likelihood that an assumption or number is true; the statistical likelihood (probability) that a random variable lays within the confidence interval of an estimate.

7. Coverage factor - the number that is multiplied by the standard uncertainty to produce an uncertainty estimate that will contain a large fraction of all values that might be obtained on a test. The coverage factor is commonly denoted as k=2 is used for 95.45% coverage and k=3 for 99.7% coverage. The drug unit uses a coverage factor (k=2) used to estimate a 95.45% level of confidence that the weights measured fall with our uncertainty window. This window varies depending on the type and readability of the respective balances.

8. Cystolithic trichomes - the claw shaped hairs found on the top side of the marijuana leaf; the simultaneous presence of these bear claw-shaped trichomes on the upper surface and the fine, slender non-cystolithic trichomes on the lower surface of the leaves is a characteristic of cannabis.

9. Expanded Uncertainty – The expanded uncertainty is the combined standard uncertainty (or standard uncertainty, if there is only one component), multiplied by the coverage factor.

10. General operating parameters - the general specifications for the method to indicate the procedure used. Should be enough so that a reviewer can locate the method file in the archive.

11. Generic name - A name that is not or does not include a trademark or brand name. The official nonproprietary name of a drug, under which it is licensed and identified by the manufacturer.

12. Grid method or test tube method– methods of weighing multiple samples at one time where a tared vessel or multiple tared vessels are placed on the balance and samples are added and then the weight is recorded. These techniques are commonly used to minimize the uncertainty of measurement.

13. Hashish - is a preparation of marijuana composed of the compressed stalked resin glands called trichomes collected from the cannabis plant. Hashish is often a paste or rock-like substance and can have varying hardness and pliability. Its color is most commonly light to dark brown, but can vary toward green, yellow, black, or red. It contains the same active ingredients but in higher concentrations than other parts of the plant such as the buds or the leaves. The psychoactive effects are the same as those of other cannabis preparations. Hashish is heated in a screened miniature smoking pipe (one-hitter, etc.), bong, vaporizer, smoked in hand-rolled cigarettes (joints) mixed with cannabis buds, tobacco, or other plant materials or aromatic herbs or cooked in foods. Analysis may reveal detached cystolithic hairs, as well as exhibit strong positive reactions to the Duquenois-Levine test and strong reactions to the TLC over sprays. GC/MS may be necessary.

14. Hash Oil - an evaporated solution of THC and various other compounds produced by a extraction of marijuana; traditionally a dark, viscous liquid; can be added to cigarettes, pipes, used in bongs or water pipes, added to food. Analysis generally will reveal no plant features, and will exhibit positive reactions to the Duquenois-Levine and TLC over sprays. GC/MS will be necessary to confirm the presence of at least one cannabinoid.

15. Homogeneous - of the same nature or kind; uniform in structure or composition.

16. Liquefaction point – the point at which a sample is completely melted; the end point of the melting process.

17. Marijuana seeds - The fruit of the marijuana plant is an achene; a single seed with a hard shell, ellipsoid, slightly compressed, smooth, about 2-5 mm long, generally brownish and mottled. The fruit is commonly regarded as a seed.

18. Mass-to-charge ratio (m/z) - the mass number of an ion divided by its charge, a dimensionless quantity used in mass spectrometry; the measurement of the sample mass as a ratio to its ionic charge.

19. Multiple unit population-a group of items that are similar in appearance, size, and composition.

20. National Institute of Standards and Technology (NIST) – A national bureau of standards and testing that sets guidelines for standards and measurement.

21. Nonproprietary name - The chemical or generic name of a drug, chemical, or device, as distinguished from a brand name or trademark.

22. Onset Point - the point at which a sample begins to melt.

23. Perfluorotributylamine (PFTBA) – the calibration material used for tuning the GC/MS instruments.

24. Primary Reference Material - a verified reference material used in the identification of substances from a verifiable source.

25. Proper/appropriate identifiers - case number, item number, etc. some means of identifying the sample and keeps it distinguishable from other items.

26. Proper scales for identification - rulers, some way of relating size (scale) to the viewer.

27. Polymorphism - when a substance can exist in multiple crystalline forms

28. Readability - the level to which a balance can read accurately.

29. Reference Material Testing Record – The records of the authentication and/or verification of reference materials used for drug identification. See also “Standard Testing Record”.

30. Regioisomeric - isomeric forms of a substance where the substances have the same molecular weight, but the atoms are attached at different places. Some spectra will be very similar.

31. Representative sample - a sample taken from an item of evidence that represents the contents of the evidence exhibit.

32. Residue - if any item weighs less than 0.04 g, or cannot be weighed, or the total uncertainty results in a measurement that is zero or negative, the item may be described and reported as a residue.

33. Secondary Reference Material - a verified, or previously analyzed, material that can be used in the identification of substances, but whose source may not be verifiable. This may include samples taken for demonstration purposes, i.e. previously identified case materials, etc.

34. Standard Testing Record - After January 1, 2011, these are named “Reference Material Testing Records”.

35. Ten Basic Spectral Colors suggested for use in Spot Tests - red, orange, yellow, green, blue, violet, pink, brown, gray, and black are suggested for color (spot) test interpretation.

36. Trade name - A name used to identify a commercial product or service, which may or may not be registered as a trademark. Also called *brand name*.

37. Uncertainty- The estimated amount or percentage by which an observed or calculated value may differ from the true value.

38. Verification - checking a piece of equipment, method or reagent to verify that it is working correctly.

APPENDIX 4
REAGENT PREPARATION MANUAL

Reagents, such as those used on chemical color (spot) testing, are used directly in testing procedures and are subject to quality control testing procedures. Reagents may be purchased, but more commonly are prepared by combining chemicals. Chemicals and/or materials used to make reagents are generally purchased from reputable chemical supply companies, such as Fisher Scientific and Sigma-Aldrich.

Reagents shall be verified with a known reference material at the time of preparation and subsequently on a monthly basis at a minimum. Exceptions are infrequently used Spot Test reagents that shall be verified with a reference material at the time of use. The preparation and monthly verification shall be recorded on the Reagent Preparation and Verification log. Bottles containing the reagents shall be labeled with the date of preparation and the initials of the forensic scientist who prepared the reagent. The Reagent Preparation and Verification log shall include the date of preparation (and subsequent monthly verification), the initials of the forensic scientist who prepared and verified the reagent, the method of verification, and the source and lot number of the reference material used to verify the reagent.

Chemicals are not used directly in testing procedures and are not subjected to the same quality control testing procedures as are reagents. Chemicals may be in dry (e.g. sodium bicarbonate), or liquid (e.g. chloroform) form. Chemicals may be used to make chemical solutions (e.g. sodium hydroxide is used to make 0.45N sodium hydroxide solution). Generally these materials can be concluded to be free from drug contamination through means such as TLC solvent blanks, GC/MS solvent blanks or even the recognition that the same source of chemical was used indirectly for separate and independent case samples resulting in unlike drug types identified or indicated.

Chemical and reagent containers shall be dated and initialed when received and also when first opened.

Chemical solutions, such as 0.5N H_2SO_4 shall be marked with the date of preparation and the initials of the forensic scientist who prepared the solution. The preparation date and initials of the forensic scientist shall be documented on the Reagent Preparation and Verification log. At the time of preparation, acid and base solutions shall be verified as either acidic or basic with the use of pH paper.

The method of verification for chemical color (spot) testing reagents requires combining the reagent with a known reference material and observing the resulting chemical color reaction. A reagent that produces the expected color reaction when combined with the known reference material is considered verified and the entry recorded on the Reagent Preparation and Verification log documents the satisfactory performance for the reagent.

TLC spray reagents, such as Fast Blue BB and iodoplatinate spray are exempt from the monthly verification entry requirement on the Reagent Preparation and Verification log. These

spray reagents are in effect verified during each use based upon their satisfactory reaction to known reference materials included on the TLC plate.

The following color test reagents, TLC reagent sprays and chemical solutions are prepared using the following or appropriately proportional procedures. A recommended reference material is included to verify the color (spot) testing reagents' performance; however, any reference material that reacts with the reagent to produce a known and expected reaction may be substituted.

Color (spot) Test Reagents

1% Cobalt Nitrate Reagent (for GHB)

1 gram of cobalt nitrate dissolved in 100 ml of distilled water

Verify using GHB (a pink-to-violet color indicates GHB)

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 300, London, Pharmaceutical Press, 2004.

Cobalt Thiocyanate Reagent (for Cocaine)

Cobalt Thiocyanate 2% by weight in water
OR
6.8 grams of cobalt chloride
4.3 grams of ammonium thiocyanate
Dissolve in 100 ml of distilled water.

Verify using Cocaine HCl (a blue precipitate indicates Cocaine).

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 294, London, Pharmaceutical Press, 2004.

Forensic Science Handbook, Volume II, 2nd edition, page 136, editor Richard Saferstein, 2002.

Cobalt Thiocyanate, Modified (for Ketamine HCl)

Add 0.1N sodium hydroxide to sample,
Add Cobalt thiocyanate reagent (Ketamine HCl produces violet color reaction)

Verify with Ketamine HCl

Reference: Modified Cobalt Thiocyanate Presumptive Test for Ketamine Hydrochloride, Morris,J., J. Forensic Sci, January 2007 Vol. 52, No.1.

Validation of Modified Cobalt Thiocyanate Test for Ketamine HCl, Curry, A. (Indiana State Police), August 2007

Supplemental Validation of Modified Cobalt Thiocyanate Test for Ketamine, Ballard, T. and Roskowski, D. (Indiana State Police), June 2009.

p-Dimethylaminobenzaldehyde (p-DMAB) Reagent (for Indoles, LSD)

Solution A: 5 grams of p-Dimethylaminobenzaldehyde in 500 ml of Methanol.
Solution B: Concentrated HCl

Verify using LSD (a violet color indicates LSD)
Or Verify using Procaine or Benzocaine (produces an intense yellow color)

Solutions A and B can also be combined prior to use.

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 284, London, Pharmaceutical Press, 2004.

Forensic Science Handbook, Volume II, 2nd edition, page 144, editor Richard Saferstein, 2002.

Dille-Koppanyi Reagent (for Barbiturates)

Solution A: 0.1 gram of Cobaltous Acetate
0.2 ml of glacial Acetic Acid
100 ml of Methanol

Solution B: 5% Isopropylamine (base) in Methanol by volume
(5 ml Isopropylamine base and 95 ml Methanol)

Verify using Phenobarbital or known barbiturate (produces blue-violet color)

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 284, London, Pharmaceutical Press, 2004.

Diphenylamine Test Reagent (for Alkyl Nitrites)

Mix 0.5 gram of diphenylamine in 20 ml of water and dilute to 100 ml with concentrated sulfuric acid.

Verify with alkyl nitrite (produces blue color)

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 284, London, Pharmaceutical Press, 2004.

Diphenylcarbazone Reagent (for Barbiturates, Glutethimide)

0.1% Diphenylcarbazone (by weight) in Methanol

Verify with Barbiturate or Glutethimide.

Reference: The Analysis of Controlled Substances, Cole. M., W. Sussex, England, Wiley, 2003, p.143.

Duquenois-Levine Reagent (Modified) (for Cannabinoids)

Solution A: 5.0 ml of Acetaldehyde
8.0 grams of Vanillin
400 ml of Methanol

Solution B: Concentrated Hydrochloric Acid

Solution C: Chloroform

Add 2-3 drops of solution A and 2-3 drops of solution B (concentrated Hydrochloric Acid). After purple/violet color develops, add 3-5 drops of solution C (Chloroform). A positive test for cannabinoids is when purple/violet color extracts into the Chloroform layer.

Verify with THC or secondary Marijuana reference material.

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 285, London, Pharmaceutical Press, 2004.

Forensic Science Handbook, Volume II, 2nd edition, page 168, editor Richard Saferstein, 2002.

The Identification of Marijuana, Thornton (University of California, Berkeley) and Nakamura (Bureau of Narcotics and Dangerous Drugs), J. Forensic Sci. Soc, (1972), 12, 461.

5% Ferric Chloride Reagent(for GHB, phenolic compounds)

5 grams of Ferric Chloride
100 ml of distilled water

Verify with GHB

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 285, London, Pharmaceutical Press, 2004.

10% Ferric Chloride Reagent (for Opium / Meconic Acid)

Dissolve a small amount of suspected opium in distilled Water

Add 3-5 drops of 10% (by weight) Ferric Chloride, red color produced
(10 grams of Ferric Chloride diluted to 100 ml with distilled Water)

Verify with Opium

Reference: SWGDRUG monograph of Opium.

Froehde's Reagent (for opiates)

0.25 grams of Molybdic Acid or Sodium Molybdate
50 ml of concentrated Sulfuric Acid (Hot)

Verify with Codeine or Morphine (produces green color reaction)

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 286, London, Pharmaceutical Press, 2004.

GHB Test # 3:

Bromocresol green: 0.03 gram bromocresol green in 100 ml 4:1 methanol: water, pH adjusted to 7.0 with 0.1 N NaOH using pH meter.

Methyl orange: 0.01 gram methyl orange in 100 ml methanol
pH adjusted to 7.0 with 0.1 N NaOH using pH meter.

Bromocresol green and methyl orange are mixed in a 1:1 ratio, then the combined reagent is mixed with **modified Schweppes reagent** in a 3:1 ratio.

Verify with GHB

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 300, London, Pharmaceutical Press, 2004.

GHB validation study 12/6/04, Nickless, R. (Indiana State Police)

Griess (Modified) Test for Nitrites

Solution A: 0.5 g sulfanilic acid in 100 ml distilled water

Solution B: 0.28 g α-naphthol in 100 ml MeOH

Mix solutions A and B together.

1) Add several drops of reagent to suspected nitrite
2) Add 1 drop acetic acid (orange color indicates the presence of nitrites)

Reference: AFTE Journal, Volume 22, Number 3, July 1990, Microgram, Volume XII, Number, (March 1979)

Griess Test for Nitrites Validation Study, Sturgeon, K. (Indiana State Police), July 2009.

Mandelin's Reagent (for aromatics with sat'd ring with one N-atom)

1 gram of Ammonium Vanadate in 100 ml of concentrated Sulfuric Acid

Verify with Morphine (produces blue-gray) or Amphetamine (green to dark green)

Reference: Forensic Science Handbook, Volume II, 2nd edition, page 166-168, editor Richard Saferstein, 2002.

Marquis Reagent (for amphetamines, opiates)

5.0 ml of 37% Formaldehyde

Dilute to 100 ml with concentrated Sulfuric Acid

Verify with an amphetamine-like substance (Methamphetamine or Amphetamine) (produces orange color reaction)

OR verify with opiate (Codeine, Heroin, or Morphine) (produces violet color)

Reference: Forensic Science Handbook, Volume II, 2nd edition, page 166-169, editor Richard Saferstein, 2002.

Clarke's Analysis of Drugs and Poisons, 3rd edition, page 289-291, London, Pharmaceutical Press, 2004.

Mecke's Reagent (for Opiates, etc.)

0.25 gram of Selenious Acid

25 ml of concentrated Sulfuric Acid

Verify with an opiate (Codeine, Morphine, or Heroin) (an immediate blue or green color is indicative of opiates).

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 289-292, London, Pharmaceutical Press, 2004.

Forensic Science Handbook, Volume II, 2nd edition, page 167-169, editor Richard Saferstein, 2002.

Schweppes reagent: (Modified) (for GHB)

Solution A: 2 grams dextrose in 20 ml water
Solution B: 2.4 grams aniline hydrochloride in 20 ml ethanol.

Mix both solutions together and dilute to 80 ml total volume with methanol.

Verify with GHB (a dark green color indicates GHB) (GBL gives a yellow-orange)

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 300, London, Pharmaceutical Press, 2004.

GHB validation study 12/6/04, Nickless, R. (Indiana State Police)

Scott (Ruybal) Test (for Cocaine)

Cobalt Thiocyanate Reagent + glycerine (1:1) (turns blue with Cocaine)
Add HCl, blue color disappears & pink solution develops
Add Chloroform, Cocaine produces intense blue color

Verify using Cocaine HCl

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 294, London, Pharmaceutical Press, 2004.

Forensic Science Handbook, Volume II, 2nd edition, page 136, editor Richard Saferstein, 2002.

Silver Ammonio-Nitrate Reagent (for Ascorbic Acid – Vitamin C)

Dissolve 2.5 gram of silver nitrate in 80 ml of distilled water

Cautiously add dilute ammonium solution until the precipitate first formed is nearly dissolved;

Allow to stand, decant the clear liquid, and add it to sufficient water to produce 100ml.

Verify with Ascorbic Acid (produces a silver-colored metallic looking reaction)

Reference: Isolation & Identification of Drugs, E.G.C. Clarke, Volume 1, page 805, Pharmaceutical Press, London, 1974.

Sodium Nitroprusside (Modified) (for secondary amines)
(aka Sodium Nitroferricyanide) (Simon's test)

Solution A: 0.25 grams Sodium Nitroprusside (Sodium Nitroferricyanide)

25 ml of distilled Water
2.5 ml of Acetaldehyde

Solution B: 0.5 gram of Sodium Carbonate
25 ml of distilled Water

Verify with Methamphetamine for secondary amine (a dark blue color indicates a secondary amine).

Reference: Clarke's Analysis of Drugs and Poisons, 3rd edition, page 295, London, Pharmaceutical Press, 2004

Tannic Acid Test (for Caffeine)

Dissolve small amount of Tannic Acid in 1-3 ml of distilled Water
(or 0.2 grams of Tannic Acid in 30 ml of distilled Water).

Drop small amount of pulverized sample onto top of solution.

Verify with Caffeine (produces white trails as sample falls through solution)

Reference: Analysis of Phentermine/Methamphetamine/Ephedrine/Caffeine Mixtures by GC/MS, R. Martin Smith, Wisconsin Department of Justice Crime Laboratory Bureau, Microgram, Volume IX, No. 4, April 1976.

Tannic Acid as a Field Test for Caffeine, Hueske, EE.; Microgram, Vol. XV, No. 9, September, 1982, p. 158.

Weber Test (for Psilocyn)

Dissolve 0.01 gram of Fast Blue B (o-dianisidine, tetrazotized) in 10 ml of Water.

Add 2 to 3 drops of reagent to sample of mushrooms
(the solution will turn red in the presence of Psilocyn)

Add 1 to 2 drops of concentrated HCl to solution
(turns from red to blue if Psilocyn is present.)

Verify with Psilocyn or a confirmed Psilocybic mushroom sample. (See above reactions.)

THC or cannabinoids can also be used if other sufficient material is not available. The reaction produced by THC or other cannabinoids may be red, purple or orange, depending on the substance used.

Reference: The Weber Test: A Color Test for the Presence of Psilocin in Mushrooms, Garrett, A.S., Clemens, S.R., Gaskill, J.H. SWAFS Journal, Vol. 15, No. 1, April, 1993, pp.44-45.

Weber Test; Garrett, Allen; Clemens, Steven and Gaskill, James. Weber State College, Laboratory of Criminalistics, Ogden, Utah. (Found in Drug Unit Resource Manual – Tryptamines Vol 2)

Weber Color Test, Koppenhaver, D., ISP Filter Paper (in-house publication), (circa 1996).

Thin Layer Chromatography Solvent Systems

Thin Layer Chromatography is generally conducted using covered glass chambers with a variety of solvents making up the mobile phase. Generally the chamber can support approximately 50 ml of solvent. The following solvent systems are routinely used and have been found over several years to provide suitable separation of components in mixtures to allow for indications of drugs present in samples. In addition, references such as the Isolation and Identification of Drugs by E.G. C. Clarke Volume 1, Clarke's Analysis of Drugs and Poisons 3rd edition and other related references provide an extensive listing of potential TLC solvent systems and visualization reagents. The list below includes, but is not limited to, the recommended and commonly used solvent systems. Several of these solvent systems and visualization reagents have been in use by the Indiana State Police Drug Unit for over 25 years.

Suspected Marijuana TLC system:

Toluene (Plates should be sprayed with Diethylamine prior to development to improve separation)

General Unknowns:

Methanol: NH_4OH (100:1.5) (This system is commonly referred to in Clarke's references as T1 and TA systems)

Chloroform: Methanol: Acetic Acid (Glacial) (75:20:5) (This system has been in use prior to January 1974)

Reference: Thin Layer Chromatography 2nd Edition, Randerath, K., Academic Press, New York and London, 1962, p 101.

Suspected LSD Unknowns:

Acetone

Acetone: NH_4OH saturated $CHCl_3$ (9:1)

Suspected Psilocyn/Psilocybin

Methanol: NH_4OH (100:1.5)

Chloroform: Methanol: Acetic Acid (Glacial) (75:20:5)

Chloroform: Methanol (4:1)

N-Butanol: Water: Acetic Acid (Glacial) (2:1:1)

Suspected Barbiturates and Hypnotics

Chloroform: Acetone (9:1)

Water: Methanol (1:1)

Suspected Benzodiazepines

Methanol: NH_4OH (100:1.5)

Chloroform: Methanol: Acetic Acid (Glacial) (75:20:5)

Chloroform: Acetone (80:20) or (9:1)

Cyclohexane: Toluene: Diethylamine (75:15:10)

Suspected Steroids

Chloroform: Ethyl Acetate (4:1)

Chloroform: Acetone (9:1)

TLC Spray Reagents

p-Dimethylaminobenzaldehyde Spray Reagent

5 grams of p-Dimethylaminobenzaldehyde
500 ml of Methanol
50 ml of concentrated Hydrochloric Acid

Or
p-DMAB color test stock solution (Solution A): HCl (Approximately 10:1)

Dragendorff Spray Reagent

Solution A: 0.57 gram of Bismuth Subnitrate
78.6 ml of glacial Acetic Acid
100 ml of distilled Water

Solution B: 14.29 grams of Potassium Iodide
312.2 ml of Distilled Water
Mix Solution A and B to prepare 500 ml of reagent

or

Solution A: 2.0 grams of Bismuth Subnitrate
25 ml of glacial Acetic Acid
100 ml of distilled Water

Solution B: 40 grams of Potassium Iodide
100 ml of distilled Water

Mix 10 ml of each of Solutions A and B, add 20 ml of glacial Acetic Acid, and add 100 ml of distilled Water. (Prepare mixture fresh as needed)

Ethanol/Sulfuric Acid Spray Reagent

20 ml of Ethanol
5 ml of concentrated Sulfuric Acid

Fast Blue B Spray Reagent or Fast Blue BB Spray Reagent

Small amount of powder dissolved in distilled Water
(Approximately 1% Fast Blue BB solution in distilled water)

Iodoplatinate Spray Reagent (Acidified)

0.25 grams of Platinic Chloride (Chloroplatinic Acid)
5.0 grams of Potassium Iodide

Dilute to 100 ml with distilled Water
Add 2.0 ml of concentrated Hydrochloric Acid

Mercurous Nitrate Spray Reagent

Saturated solution of Mercurous Nitrate in distilled Water

Ninhydrin Spray Reagent

0.5 gram of Ninhydrin
1.0 ml of glacial Acetic Acid
100 ml of Isopropyl Alcohol

or

2% Ninhydrin in Acetone
(2 grams of Ninhydrin in 100 ml of Acetone)

Potassium Permanganate Spray Reagent

2.0 grams of Potassium Permanganate
5 drops of Phosphoric Acid

Dilute to 100 ml with distilled Water

or

1 gram of Potassium Permanganate
Dilute to 100 ml with distilled Water

Acid and Base Solutions

Concentrated Hydrochloric Acid = 12 Normal = 12 Molar

2.8N Hydrochloric Acid (Check with pH paper)

116.5 milliliters of concentrated Hydrochloric Acid
Add to distilled Water for final volume of 500 ml.

Saturated Sodium Hydroxide = 17.3 Molar = 17.3 Normal

0.45 N Sodium Hydroxide (Check with pH paper)

26.01 ml of saturated Sodium Hydroxide (saturated in distilled Water)
Dilute to 1.0 liter with distilled Water

or

18 grams of Sodium Hydroxide
Dilute with 1000 ml (1.0 liter) of distilled Water

Concentrated Sulfuric Acid = 36 Normal = 18 Molar

0.5 N Sulfuric Acid (Check with pH paper)

13.9 ml of concentrated Sulfuric Acid
Add to distilled Water for final volume of 1.0 liter

APPENDIX 5
Instrument Preventive Maintenance

Preventive maintenance is performed on analytical equipment to ensure the system continues to perform properly. This is accomplished by inspecting, testing and/or cleaning the equipment at specific intervals. All preventive maintenance shall be documented in the appropriate instrument's maintenance log. The maintenance schedules below represent the maximum maintenance intervals.

Each instrument has a primary operator that is responsible for the scheduling of calibration, conducting verifications and maintenance of the instrument. In the absence of the primary operator, another analyst shall be assigned these duties.

The maintenance schedules for balances, ultraviolet spectrophotometers, polarimeters, melting point apparatus, and FTIR instruments are located within their individual Test Methods section.

Microscopes

Microscopes shall be cleaned as needed. If a microscope fails to perform properly or is in need of repair, the appropriate personnel shall be notified.

Gas Chromatograph/Mass Spectrometer and Gas Chromatograph-Infrared Spectrophotometer

The GC/MS and GC-IR instruments shall be serviced as needed. When one of the instruments cannot pass the calibration and quality control checks as per Drug Unit Test Methods, it shall be serviced by a qualified examiner or certified technician. The service shall be documented in the maintenance log for the instrument. Before being placed back into service, the instrument shall pass all calibration and quality control checks.

The instrument shall be cleaned as needed. The filament, column, and seals shall be replaced as needed. The column insert, auto-sample syringe and septum shall be replaced as needed.

For GC/MS, if maintenance is performed on the GC, which necessitates the instrument being vented, a blank and test mix shall be run before putting the instrument back into service. If maintenance is performed on the MS, then a tune, blank, and test mix shall be run before the instrument is placed back into service. If a filament is switched without venting the instrument, only a tune is required to put the instrument back into service.

For GC-IR if maintenance is performed on the GC that requires removing the column from the inlet or butt connector, a blank and test mix shall be run before putting the instrument back into service. If maintenance is performed on the IR, then a voltage check, noise check, and polystyrene reference material check shall be run before the instrument is placed back into service.

APPENDIX 6

WASTE DISPOSAL PROCEDURES

Non-hazardous Chemical Waste

Not all waste materials and chemicals in a laboratory are hazardous waste. For Indiana State Police Laboratory Division, these include paper and plastic trash, empty containers, broken glass, non-hazardous liquid and solid wastes, GC/MS vials, and color/spot testing waste from spot plates.

Solvents used in extractions to recover drugs for further analysis are evaporated in a fume hood.

> Example: Chloroform used in extraction from 0.45N Sodium Hydroxide to purify Hydrocodone from a mixture with acetaminophen is evaporated to recover Hydrocodone for further analysis.

Chemicals from color/spot testing and clean-up of spot plate may be disposed of through flushing into a sink drain connected to a sanitary sewer with at least twenty (20) volumes of water for each volume of waste.

GC/MS vials containing small amounts of solvent can be disposed of in the “broken glass” box for final disposal in “normal” trash.

Empty glass containers and broken glass are collected in “broken glass” boxes. Empty containers as defined by EPA and IDEM are described in the Laboratory Waste Management Program. After the “broken glass” boxes are full, they shall be sealed for final disposal in “normal” trash.

Empty aerosol cans may be disposed of in “normal” trash. To be considered empty, aerosol cans must contain no propellant and no product, and must be at atmospheric pressure.

Non-hazardous liquid and solid wastes may be processed for disposal down a sink drain or in “normal” trash as outlined in the Laboratory Waste Management Program. A list of non-hazardous chemicals suitable for drain or trash disposal is included as an appendix in the Laboratory Waste Management Program. You may dispose these types of solid chemicals in normal trash if the containers are tightly capped and of good integrity.

If you are unsure whether or not you should dispose of a material as a non-hazardous waste, then it should be handled as a hazardous chemical for waste disposal.

Acid and Base Disposal

Acidic and alkaline (basic) chemical wastes are classified as hazardous waste if the pH is less than or equal to 2 or greater than or equal to 12.5. If the acid or alkaline waste ONLY has characteristics of corrosivity and is NOT a listed waste, it may be neutralized to within a pH range of 5 to 9 before disposal to a sanitary sewer. Neutralization can be incorporated in the analysis procedure.

Neutralized acid and alkaline waste shall be flushed with at least twenty (20) volumes of water for each volume of waste.

Acid and Base Neutralization Procedures

These procedures explain the disposal of concentrated solutions of acids, such as hydrochloric, nitric and sulfuric acid, and bases such as ammonium hydroxide and sodium hydroxide.

Caution: vapors and heat are generated during neutralization.

You are not required to neutralize any wastes yourself. If you choose to neutralize and dispose of these materials yourself, please adhere to the following.

- Perform all steps slowly.
- Keep containers cool while neutralizing.
- **Acid neutralization:** While stirring, add acids to large amounts of a cold solution of aqueous base (sodium carbonate, calcium hydroxide, or 8 M sodium hydroxide).
- **Base neutralization:** First add the base to a large vessel containing cold water. Slowly add a 1 M solution of HCl.
- Neutralize concentrated acid and base solutions to within a pH range of 5 to 9, and then flush them into the sanitary sewer with at least 20 volumes of water for each volume of waste.
- **If necessary, allow the contents to react for at least twenty-four hours to obtain a stable pH and to dissipate the any heat associated with the neutralization reaction. The container should not be hot and the contents should not be smoking.**

Hazardous Chemical Waste

Laboratory personnel are not responsible for final classification of waste chemicals for hazardous waste manifests, yet must be generally aware of waste classification criteria to determine if a chemical is hazardous or non-hazardous for disposal. The classifications for hazardous wastes are: F-list, K-list, U-list, P-list, and characteristic wastes. Information is included in the Laboratory Waste Management Program to classify potential hazardous chemical wastes.

F-list waste – These are non-specified source waste. This includes all spent solvent mixture/blends containing, before use, a total of 10% or more (by volume) of one or more of the solvents listed in F001, F002, F003, F004, and F005

K-list waste - **this list does not apply to Indiana State Police Laboratories**. It includes certain waste from specific industries, such as petroleum refining or pesticide manufacturing.

U- and P-list waste - (discarded and unused commercial chemical products) U and P list waste include specific commercial chemical products in an unused or "virgin" form.

> **Virgin chemicals** – a chemical that has not been previously used or consumed, or subjected to processing other than for its original production.
> **P-list chemicals are classified as acutely hazardous waste**, and are subject to a 1 kg limit for accumulation quantity.

Characteristic hazardous waste groups are classified by characteristics of ignitability, corrosivity, reactivity, and toxicity.

Drug Unit procedures for collection, storage and disposal of hazardous waste

The proper way to collect and store hazardous waste is through use of hazardous waste containers in a Satellite Hazardous Waste Accumulation area until full. Transfer the full container to a Central Hazardous Waste Accumulation area for disposal by a contracted chemical waste disposal vendor.

A minimum of three satellite hazardous waste collection containers shall be available for use in the Drug Unit.

1. Chlorinated waste including chloroform, chloroform mixtures (i.e. chloroform, methanol and acetic acid thin layer chromatography system mixture), etc.

2. Flammable wastes including methanol, acetone, pentane, hexane, petroleum ether, toluene, flammable organic chemical mixtures, etc.

3. Oxidizers including iodoplatinate and potassium dichromate

Organic chemicals used as a rinse in cleaning glassware shall be collected as either chlorinated wastes or flammable waste.

> Example: Chloroform rinse of glassware shall be collected as chlorinated hazardous wastes. Methanol rinse of glassware shall be collected as flammable hazardous wastes.

Solvents used in extractions that are not evaporated to recover drugs for further analysis shall be processed as hazardous waste.

> Example: Mushroom extractions using chloroform from acid, followed by making the aqueous solution basic and extracting with chloroform to extract psilocin. Chloroform from the acidic extraction shall be handled as chlorinated hazardous waste. Chloroform from the basic extraction will be evaporated to recover psilocin.

Chemicals in color tests (i.e. Duquenois Levine) conducted in a test tube shall be processed as chlorinated hazardous waste.

Full or partially empty aerosol cans shall be collected for disposal as hazardous waste in a satellite container labeled as "Aerosol Cans" for hazardous waste disposal. IDEM and EPA regulate all partially empty spray cans as hazardous waste, because they may still contain chlorinated solvents, flammable material or toxic substances. **Do Not** discard partially empty spray cans in the trash. **Do Not** puncture any aerosol cans.

Dilution of hazardous chemical wastes and disposal in the sink drain is not the proper way to dispose of hazardous waste.

If a spill occurs, the chemical in the spill and the materials used to clean up the spill are considered to have the same hazard classification. Spill clean-up materials are not to be thrown in the “normal” trash. These materials are to be properly disposed of as hazardous waste. Procedures in the Laboratory Chemical Spill Management Program shall be used for spill clean-up and disposal.

Hazardous Waste Containers

1. Each laboratory and/or Unit must supply their own containers
2. For liquid wastes, the amber 4 liter solvent bottles are preferred because they are non-recyclable and are compatible with most types of waste.
3. All containers must be in good condition and compatible with their waste contents. The original container the chemical came in is usually the best container for chemical waste.
4. All containers must have securely fitting lids or caps.
5. Funnels shall be removed and not left in waste containers.
6. Hazardous waste container shall be marked “hazardous waste.”
7. A log of chemical waste contents, quantities, dates wastes were added and initials of waste generator shall be listed on the label or an attached tag.
8. Containers shall be stored with a closed lid or cap.

Liquid Hazardous Waste Containers

1. Leave 10% headspace (volume left at top of container) in case of expansion due to temperature.
2. Do not pour hot liquids into hazardous waste bottles.
3. Do not combine or comingle incompatible wastes (i.e. acids and bases)
4. Provide secondary containment.
5. Any container with a capacity of less than or equal to 4 liters must have secondary containment.

Solid Hazardous Waste Containers

1. The original container is generally the “best” waste container for solid hazardous waste.
2. If original containers are not available – double bag the material and place in a sturdy cardboard box for support.
3. Do not use Biohazard bags.
4. Bags used should be trash bags.

Satellite Hazardous Waste Storage

1. Hazardous waste regulations require that the generator accumulate hazardous chemical waste in containers at or near the point of generation where waste initially accumulates until full and which is under the control of the operator who generated the waste.
2. Under no circumstances shall waste be stored down the hall and/or out of your control.

Central Hazardous Waste Storage

1. Full hazardous waste containers shall be marked with the accumulation date (date the satellite waste container was completely filled with the hazardous waste, not the date the collection of hazardous waste began in the satellite container).
2. Move the full waste container to the Central Hazardous Waste Accumulation Storage area within three days after being filled to capacity.
3. All waste containers must have securely fitting lids or caps.
4. Provide secondary containment, as necessary to contain spills.

Secondary Containment to Minimize Spills of Hazardous Wastes

1. Secondary containment shall be used to minimize the potential for breakage, spillage and the comingling of incompatible materials (i.e. acids and bases).

2. Plastic trays, pans, or tubs may be used.

3. Without exception, secondary containment is required for the following:
   A. All glass containers of liquid hazardous waste stored on the floor.
   B. All containers with capacity less than or equal to 4 liters of liquid hazardous waste, regardless of storage location.

4. Hazardous materials shall be segregated by hazard class and stored in separate cabinets, trays or pans.

Example – Leaks with Spill Contained in Tray

Lids or Caps on Hazardous Waste Containers

1. Lids must be securely in-place except when material is being removed or added to the container.

2. A funnel resting on the mouth of a bottle does not constitute a lid

3. Lids on waste containers must be on tight (Note: Be sure that gas producing reactions have worked to completion before transferring the material to a hazardous waste container).

4. A closed container, when tipped over, won't leak!

Example - Improper Lids [Open Containers]

1. Lid open when not in use. 2. A funnel is not a lid.

INDIANA STATE POLICE
DRUG UNIT
TEST METHODS

Labels on Hazardous Waste Containers

1. If a chemical container is reused, the original label must be defaced, removed or completely covered.

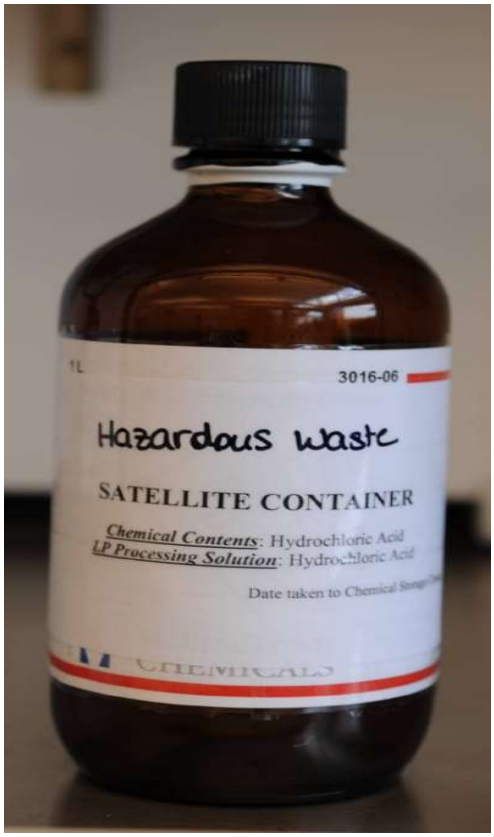
2. EPA and IDEM regulations require that the name of waste chemicals be clearly identified on the label or attached tag.

3. Chemical formulas and abbreviations such as H_2SO_4, HCl, NaOH, HOAc, and MeOH are **NOT** accepted by EPA and IDEM. Use the chemical name such as sulfuric acid, hydrochloric acid, sodium hydroxide, acetic acid and methanol.

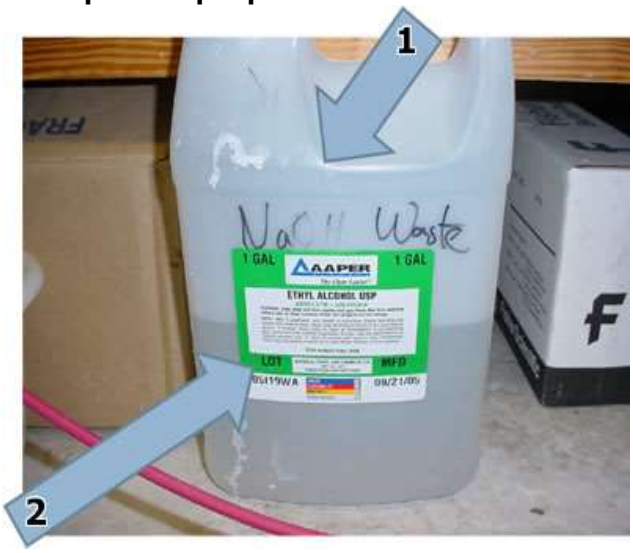
4. Hazardous waste regulations require the words "Hazardous Waste", or words which clearly identify the contents such as "Acetone Waste", be on each waste container.

5. The satellite container label must have an area where the accumulation date (the date that the container is full – NOT the date that collection began in the container) can be documented.

Example – Proper Label



Example - Improper Label



1. Chemical formulas or abbreviations are not allowed.

2. If you re-use a container for collecting hazardous waste, you must deface, cover, or remove the original label.